SLEEP REGULATION IN THE RAT: NEUROCHEMICAL MECHANISMS AND THE EFFECT OF LIGHT-DARK SCHEDULES

INAUGURAL-DISSERTATION

zur Erlangung der
Philosophischen Doktorwürde
vorgelegt der
PHILOSOPHISCHEN FAKULTÄT II
DER UNIVERSITÄT ZÜRICH

von
IRENE TOBLER-KOST
aus Teufen, AR

Begutachtet von den Herren

Prof. Dr. A.A. Borbély
Pharmakologisches Institut der Universität Zürich

Prof. Dr. R. Wehner
Zoologisches Institut der Universität Zürich

Dr. W.B. Mendelson
National Institute of Mental Health, Bethesda, USA

ADAG Administration & Druck AG

Zürich 1980

SLEEP REGULATION IN THE RAT: NEUROCHEMICAL MECHANISMS AND THE EFFECT OF LIGHT-DARK SCHEDULES

INAUGURAL-DISSERTATION

zur Erlangung der
Philosophischen Doktorwürde
vorgelegt der
PHILOSOPHISCHEN FAKULTÄT II
DER UNIVERSITÄT ZÜRICH

von
IRENE TOBLER-KOST
aus Teufen, AR

Begutachtet von den Herren

Prof. Dr. A.A. Borbély
Pharmakologisches Institut der Universität Zürich

Prof. Dr. R. Wehner
Zoologisches Institut der Universität Zürich

Dr. W.B. Mendelson
National Institute of Mental Health, Bethesda, USA

ADAG Administration & Druck AG

Zürich 1980

UNIV.
LEIDEN
BIBL.

ACKNOWLEDGEMENTS

It is a great pleasure to thank my supervisor Professor Dr. Alexander A. Borbély for introducing me to the fascinating topic of sleep. I thank him for encouraging me to pursue this research and for emphasizing to me the importance of the many small steps it takes to clarify a scientific question. Without his guidance and critical advice at all times this work would not have been possible.

I would like to warmly thank Mr. Neuhaus for his valuable help with the computer and for his expert assistance with the sleep-recording equipment; also, for his moral support.

I am very grateful to Mr. J. Bisang and Mr. K. Wüthrich for the construction and maintenance of the experimental apparatus.

For her conscientious help and devotion during the preparation of this thesis I would especially like to thank Miss K. Schwarz.

Finally I gratefully acknowledge the assistance of the many persons who offered their help and advice in various stages of this work.

This study has been supported by the Swiss National Science Foundation grant no. 3.624-0.75 and 3.254-0.77.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41507212_0071

Table of contents

Page

List of figures 5
List of tables 6
List of abbreviations 7

I INTRODUCTION 9

II METHODS 12

A Animals and substances 12

B Recording of motor activity 13

1. Method I
2. Method II

C Sleep deprivation 14

D Sleep recordings 15

1. Cable recordings 15

Operation
Electrodes
Plug
Screws
Experimental box
Pre-amplifier
Polygraph
Calibrator
Data analysis

2. Telemetry 22

Operation
Transmitter
Experimental conditions
Recording
Data analysis

E Drug application methods and effect of intra-ventricular infusion 25

1. Drug administration 26

Cannula
Surgery
Infusions
Electrocardiogram (ECG)
Infusion, sleep and ECG

2. Chronic release from implanted pellets 30

Release of subcutaneously implanted pellets

3. Cerebroventricular drug administration 31

Intraventricular administration procedure 33
Histology 34
Cerebroventricular infusion and motor activity 34
Cerebroventricular infusion and sleep 40
Feasibility
Ca-ions
Mg-ions
Experiments with artificial cerebrospinal fluid (aCSF)
aCSF-I and excess Ca
aCSF-II and aCSF-III
aCSF-II and excess Mg
Discussion 47
Post-infusion effects 49

F Chemical determinations, Bioassay 51

1. Serotonin

2. Delta Sleep Inducing Peptide (DSIP)

3. Arginine vasotocin (AVT) 51

Thinlayer chromatography (TLC)
High-Pressure liquid chromatography (HPLC)
Determination of peptide loss through adherence
Bioassay

4. Lysergic acid diethylamide (LSD and further substances) 53

G Further methods 55

1. Hypophysectomy 55

2. Aspiration of cerebrospinal fluid (CSF) 57

3. Gas chromatography (GC) determination of ether in CSF 57

III REM-SLEEP-INDUCTION BY SHORT DARK OR SHORT LIGHT PERIODS 58

A Effect in the golden hamster 60

B Effect in two rat strains 62

C Effect in hypophysectomized rats 64

IV NEUROTRANSMITTERS AND SLEEP REGULATION 65

A Serotonergic system and sleep 66

1. Serotonin: Effect of intraventricular infusion on sleep 67

2. Lysergic acid diethylamide (LSD): Effect of intravenous infusions on sleep 68

3. Discussion 68

4. Parachlorophenylalanine (PCPA) 69

Effect of cerebroventricular PCPA-administration on motor activity
Effect of PCPA on body weight

5. Effect of tryptophan (TRP) on PCPA-pretreated rats 72

Sleep
Forebrain serotonin concentration
Discussion

6. Effect of a serotonin antagonist (FU 29-245) on sleep 77

B Cholinergic system and sleep 78

1. Physostigmine 81

Telemetric pilot study
Experiments with physostigmine
Experiments with methyl-atropine and physostigmine
Discussion

2. Carbachol: Effect of intraventricular infusions on sleep 84

3. Effect of atropine pellets on motor activity 85

4. Effect of atropine pellets on sleep 86

V PEPTIDES AND HUMORAL SLEEP FACTORS 88

A Peptides

1. Experiments with Delta Sleep Inducing Peptide (DSIP) 90

Effect of DSIP on motor activity
Effect of DSIP on sleep

2. Experiments with arginine vasotocin (AVT) 98

Effect of AVT on behavior
Effect of AVT on sleep
Effect of AVT on hypothalamic serotonin

3. Discussion 101

B Humoral sleep factors 104

1. CSF from sleep-deprived rats 104

Motor activity
Sleep

VI CONCLUDING REMARKS 107

VII SUMMARY 110

VIII REFERENCES 113

List of figures

		Page
1.	Rat with transmitter for EEG and EMG recording.	11
2.	Rat with plug, cable, chronic cannula and tubing.	11
3.	Rotating cylinders for sleep deprivation.	14
4.	Electrodes and cannula in relation to the rat skull.	17
5.	Rat in cable-recording chamber.	20
6.	Rat in telemetry recording chamber.	20
7.	Transmitter.	23
8.	Screw and intraventricular cannula.	32
9.	Silicone cannula for intravenous infusions.	26
10.	Insertion of cannula into the vein.	28
11.	Release rate of atropine and theophylline from subcutaneously implanted pellets.	31
12.	Brain section indicating placement of cannula.	35
13.	Effect of intraventricular infusion on motor activity pattern. Original plot.	37
14.	Effect of intraventricular infusion on motor activity. Histogram.	38
15.	Occurrence of feeding during and after infusion of aCSF with excess Ca or Mg.	47
16.	Effect of cerebroventricular infusion of aCSF and natural CSF on sleep.	50
17.	IR-spectrum of a freshly prepared, and of a two month old LSD solution.	54
18.	Basal aspect of the rat skull.	56
19.	Frequency of REMS onset in the golden hamster during a 5 : 5 min LD cycle.	60
20.	Motor activity of two rats before and after infusion of PCPA or NaCl. Original plots.	72
21.	Serotonin concentration in the rat forebrain determined at various timepoints after injection of PCPA.	76
22.	Vigilance states and EEG power spectra in a rat recorded during a control day and one day after PCPA and TRP administration.	74
23.	Chemical structure of FU 29-245, a serotonin antagonist.	77
24.	Effect of i.p. injection of FU 29-245 on sleep.	79
25.	Spectral power density of the EEG before and after i.p. injection of FU 29-245.	80

26. Effect of intraventricular infusion of physostigmine on REMS. 83

27. Motor activity of a rat recorded continuously after the implantation of atropine pellets. 86

28. Structure of arginine vasotocin (AVT) and of Delta Sleep Inducing Peptide (DSIP). 88

29. Effect of DSIP on motor activity (11-13 days). 91

30. Effect of DSIP on motor activity (4-h post-injection period). 92

31. Vigilance states and EEG power of the delta band before and after intraventricular injection of DSIP in one rat. 93

32. Vigilance states and EEG power of the delta band after intraventricular injection or infusion of DSIP. 95

33. Effect of AVT on visually scored activity and sleep. 99

34. Effect of intraventricular injection of AVT on sleep. 100

35. Effect of CSF from sleep deprived donor rats in sleeping recipient rats. 106

List of tables

1. Concentration of ions in different types of aCSF. 36

2. Sleep parameters during infusions of different types of aCSF. 42

3. Occurrence of feeding during and after infusion of normal aCSF and aCSF with excess Mg. 45

4. Sleep states of the golden hamster during short LD-periods. 61

5. Distribution of sleep during short LD-cycles in intact and hypophysectomized albino rats and in the non-albino DA rat strain. 63

6. Sleep parameters after the implantation of atropine pellets. 87

7. Effect of DSIP on motor activity. 97

List of abbreviations

ACh	acetylcholine
aCSF	artificial cerebrospinal fluid
ACT	activity
AD	analoge digital
AM	amplitude modulated
AVT	arginine vasotocin
CNS	central nervous system
cpm	counts per minute
CSF	cerebrospinal fluid
d	diameter
depr. CSF	CSF from sleep deprived rats
DSIP	Delta Sleep Inducing Peptide
ECG	electrocardiogram
EEG	electroencephalogram
EMG	electromyogram
FM	frequency modulated
HAND	handling
INF	infusion
INJ	injection
i.p.	intraperitoneal
i.v.	intravenous
lat.	lateral
LD	light-dark
LH	luteinizing hormone
LSD	lysergic acid diethylamide
MA	monoamines
NE	norepinephrine
NREMS	non-REM sleep
PCPA	parachlorophenylalanine methyl ester
PE	polyethylene
p.o.	per os
PVC	polyvinylchloride
PVP	polyvinylpyrrolidone

REMS	REM sleep, rapid eye movement sleep
SEM	standard error of the mean
SWS	slow wave sleep
TRP	tryptophan
W	waking
5-HT	serotonin, 5-hydroxytryptamine
5-HTP	5-hydroxytryptophan

I INTRODUCTION

Two of the most basic questions in sleep research are the problem of the biological function of sleep, and of the mechanisms underlying sleep regulation. Continuous polygraphic recordings have shown that the quantity and quality of sleep are relatively constant from day to day, and can thus be used as a reliable baseline in the study of neuropharmacological agents. The neuropharmacological approach allows to dissect some of the intricate mechanisms underlying the sleep-waking cycle since some drugs may either affect sleep as a whole, or influence a specific sleep state selectively.

The discovery of a long-lasting rebound after instrumental or pharmacological suppression of sleep, paved the way for the entry of "wet neurophysiology" into sleep research. Two main paths may be discriminated: first, revival of the old theory of hypnotoxin (Piéron, 1913), which assumes that some protein or polypeptide is liberated into the blood, the cerebrospinal fluid (CSF), or brain during sleep, and attempts to characterize a hypnogenic factor: the humoral theory of sleep. Second, the neuropharmacological approach served to specify the role of putative neurotransmitters that might be involved in the regulation of sleep. This led to the development of the cholinergic and monoaminergic theories of sleep.

The two approaches which have been commonly used to study the role of transmitters in sleep are, (i) to alter as selectively as possible the activity of a given transmitter system and study the effect upon waking and sleeping; or (ii) to alter sleep and waking, and study the effect on a transmitter system. In addition to these pharmacological approaches to the problem of sleep regulation, a purely biological approach should be also mentioned,which investigates the influence of the environment. The circadian sleep-wake pattern in rodents is strikingly stable. Webb and Friedmann (1971) showed that the sleep

patterns of rats are very resistant to environmental variations. Despite these findings attesting to the stability of sleep and activity rhythms, there is increasing data that some environmental parameters may influence sleep in the rat: changes in the ambient lighting conditions (Lisk and Sawyer, 1966; Rechtschaffen et al., 1975 and Borbély, 1976), variations of the ambient temperature (Browman, 1953; Valatx et al., 1973), and changes in feeding schedules (Folk, 1954). Manipulation of these conditions have proven to be valuable tools in assessing the mechanisms of the apparently very strong inherent control system of the sleep-activity cycle.

Fig. 1: Male Sprague Dawley rat with a two-channel transmitter for recording EEG and EMG in sleep studies. From A.A. Borbély, Progress in Neurobiol. 1978, 10: 1-31.

Fig. 2: Male rat with a plug for cable recording of EEG and EMG. In addition the rat is implanted with a chronic cannula in the lateral ventricle.

Figure 1:

Figure 2:

II METHODS

A Animals and substances

Rats: Sprague Dawley Ivanovas, SIV-50 strain, Tierspital Zurich
DA rats, Inst. für Mikrobiologie, Univ. Zurich
Hamsters: Mesocricetus auratus, Inst. für Mikrobiologie, Univ. Zurich.

The animals were kept either in groups in wire mesh cages (47 x 30 x 15 cm) or individually in plexiglas cages with wire lids (37 x 16 x 15 cm). They had unlimited access to food (rat chow NAFAG, No 890, Switzerland) and water.

For telemetric sleep or motor activity registration the animals were transferred into a cylindrical plexiglas cage (40 x 20 x 80 cm). All other experiments were carried out in plexiglas cages with no top. For additional height, a plexiglas frame (30 x 20 x 35 cm) was placed in the cages.

All experiments were carried out with rats weighing approx. 280 - 320 g unless stated otherwise.

Substances

arginine vasotocin: Bachem, Switzerland; Heidelberg G.F.R.
atropine sulphate: Kantonsapotheke, Zurich, Switzerland
carbachol: Kantonsapotheke, Zurich
Delta Sleep Inducing Peptide: Kantonsspital Basel; Calbiochem, Switzerland
FU 29-245: Sandoz AG, Basel, Switzerland
glycerintripalmitate: Kantonsapotheke, Zurich
l-tryptophan: Fluka, Buchs SG, Switzerland
lysergic acid diethylamide: Sandoz AG, Basel
methyl-atropine bromide: Merck, Darmstadt, G.F.R.
Paladur: Kulzer & Co., Bad Homburg, G.F.R.
p-chlorophenylalanine-methylester: Sigma, St. Louis, U.S.A.
pentobarbital: Kantonsapotheke, Zurich

physostigmine salicilate: Fluka AG, Buchs SG
polyvinylpyrrolidone: Kantonsapotheke, Zurich
serotonin creatinine sulphate: Fluka, Buchs SG
theophylline: Kantonsapotheke, Zurich
Tween-80: Bender & Hobein AG, Zurich

B Recording of motor activity

1. Method I

For motor activity recordings the rat cage was placed on a platform resting on springs. The motor activity signal was generated by a force recorder under the cage consisting of two moving coils which served as mechano-electrical transducers. The vertical component of force changes occurring during motor activity induced proportional changes in voltage which were amplified and full-wave rectified to provide the activity signal (Borbély, 1970) which was integrated for 10-s epochs.

2. Method II

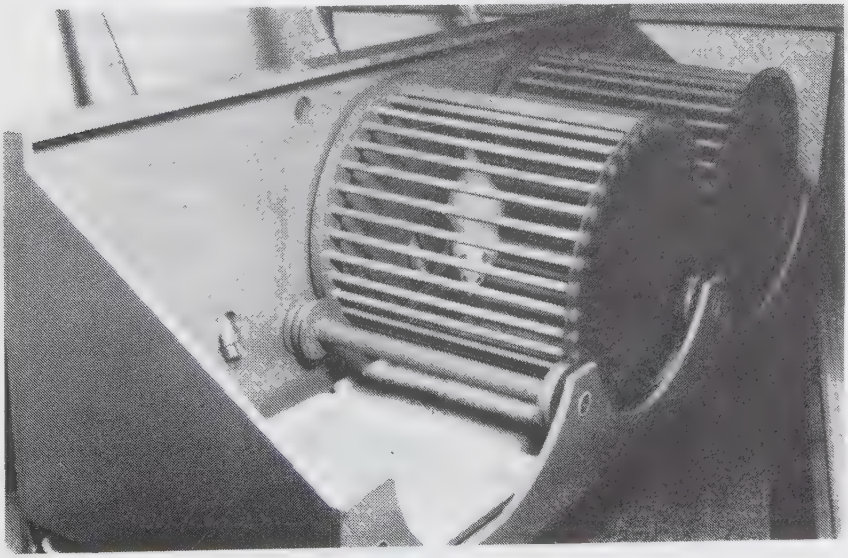
A motor activity recording system which allows simultaneous measurement of motor activity of 15 rats was constructed. The apparatus consisted of a large sound attenuated box divided into 15 compartments into which the plexiglass rat cage was placed. The animals were held under control light-dark (LD) conditions and at an ambient temperature of 22 - 25°C. Motor activity was measured with 3 piezoelectric force recorders under each cage.Vibrations of the whole box and interactions between the compartments were damped by three rubber buffers. The signal was amplified, full-wave rectified and integrated. When the integrated signal reached a defined level a pulse was generated, counted and registered, for each animal separately, by the event-recorder. At 15-min intervals the total number of pulses was stored on paper tape. The sensitivity of the system

was adjusted so that during the daytime hours minimum average of pulses was ca. 3, and during night time 25. The maximum number was 99 pulses/15 min. The transducing systems of the individual compartments were adjusted to equal sensitivity by a vibration-generator.

C Sleep deprivation

For sleep deprivation rats were placed in a rotating activity drum (rotation rate: one rotation per 45 s). Fig. 3. The drum consisted of a cylinder with regularly spaced PVC-rods (length: 30 cm; diameter: 10.4 mm; interval between rods 15.5 mm) fixed on one side to a PVC disk and on the other to a PVC ring (30 cm d), which permitted access to food and water.

Figure 3:



Rotating cylinders for sleep deprivation.

D Sleep recordings

In spite of the undoubted advantages of studying an animal in its natural environment only polygraphic recordings of the EEG in the laboratory have allowed a more detailed approach to the sleep phenomenon.
The traditional sleep recordings are obtained with cable connections. This is an adequate technique for many experimental purposes, in spite of the evidence that the animal's behavior may be influenced by the cable leads (Haupt, 1973). A sufficient number of adaptation days and adequate control sessions are necessary. Nevertheless a slight impairment of movement, as well as acoustic stimuli originating from the cable and swivel movements have to be kept in mind. Cable recordings during the active phase of the sleep-waking cycle necessitate sturdy cable leads which may further hinder the animal.
A recent and more sophisticated telemetric method makes it possible to study the long-term behavior of animals in their natural habitat, or under completely unrestrained laboratory conditions. This technique was used in some of our experiments to obtain uninterrupted recordings from unrestrained rats for several days, i.e. during both the light-phase and the dark-phase of the daily LD-cycle.

1. Cable recordings

Operation

Under pentobarbital anesthesia (Pentobarbital 1 %, 1 ml/kg i.p.) the head of the animal was fixed with two ear plugs so that the interaural line was either 5 mm (Pellegrino and Cushman, 1967) or 2.4 mm (König and Klippel, 1963) below the level of the upper incisor bar. An oval scalp section was cut away, and the skull cleaned meticulously of all skin and blood. Two holes were drilled through the scalp taking care to avoid rupturing

the dura, and electrodes were positioned on the dura over the parietal cortex and the cerebellum. Two electrodes were inserted perpendicularly into the neck muscles. Two to three screws (Fig. 8) were screwed into the skull for better fixation of the dental cement (Paladur FDI-3-II, Kulzer, Germany). The electrodes were soldered to the pins of a connector that was cemented to the skull, thus permitting cortical EEG and EMG recording via cable leads. In hamsters the connector was cemented longitudinally due to the narrow shape of the skull. Fig. 4.
Three to four days were allowed for recovery from the operation during which the animals were daily handled. Frequent determinations of body weight served to check the animal's recovery.

Electrodes

In animals without a chronic infusion cannula stainless steel electrodes were used (0.2 mm d). The tip of the electrode was bent to form a small circle (1.5 mm d) which was positioned flat on the dural surface under the skull.
In the experiments with a chronic cannula gold or platinum electrodes (0.2 mm d) were used. Both materials had the advantage that the tips could be melted to form a ball (0.7 mm d). The ball tip improved the quality of the EEG recordings, and fewer animals had to be discarded due to an injured brain surface and to artifacts in the EEG.
The electrodes were cleaned in an ultra sound bath with H_2SO_4 1m and with a soap solution, rinsed, welded to the plug and kept in ethanol.

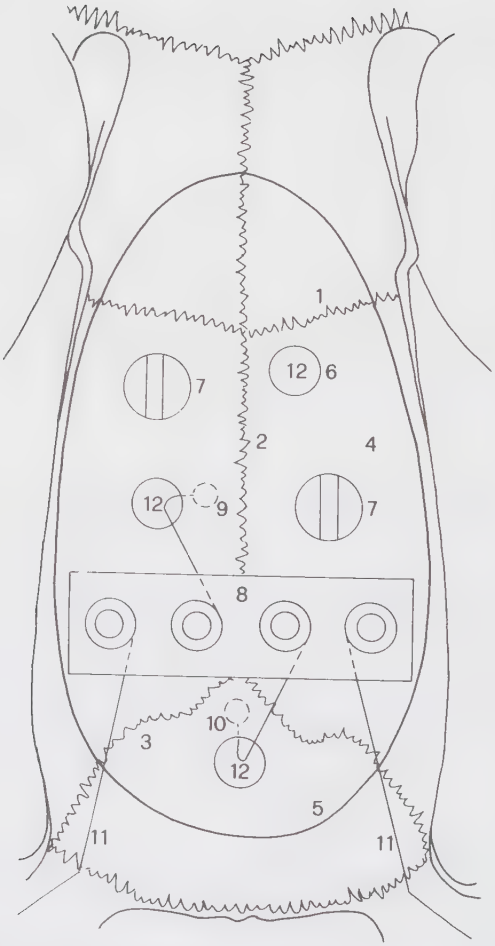
Plug

AUGAT type 508 AG/10D, 10/3 mm (Dewald AG Zurich, Switzerland).

Screws

Fig. 8
Gold plated brass screws, Parker type, 0.5 mm thread.

Figure 4:



Electrodes and cannula in relation to the skull

1. coronal suture
2. sagittal suture
3. lambdoid suture
4. parietal bone
5. interparietal bone
6. cannula (lat. ventricle)
7. screws
8. plug
9. EEG electrode (cortex)
10. EEG electrode (cerebellum)
11. EMG electrodes
12. drill holes

Experimental box

Once the animal regained the pre-operation body weight it was transferred to a sound-attenuated chamber with a constant white noise and with a constant LD-cycle. Ambient temperature ranged from 21° to 26°C, although during one single experiment it never varied by more than 3°C.
Light was provided by daylight-type neon tubes, with a light intensity at the cage level depending on the direction, amounting to 40 - 250 lm/m^{2}.
Heating of the box by the light source was avoided by a fresh water system circulating through a mantle around the neon lights. Under these conditions the animals were adapted to the cable for 2 - 5 days.
To avoid cable gnawing the cable was connected to a swivel, and cable and swivel were attached to a beam. (Fig. 5).
The beam was counterbalanced by weights, so as to reduce inertia and allow an immediate adjustment to the animal's movements. The pull on the rat (10 - 15 g) was just enough to prevent the cable from sagging. When the rat reared in the cage the cable stayed taut. The system allowed the animal to sleep in a natural posture with its head tucked under the body.
All cable recordings were carried out during the light period of the LD-cycle.

Figure 5: (left)

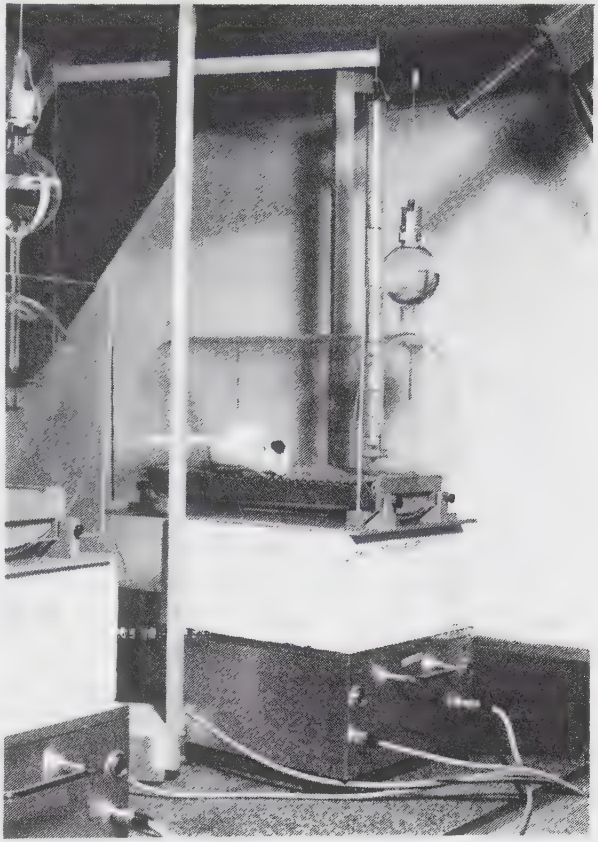
Sleep recording box. A rat with chronically implanted EEG and EMG electrodes is attached to cables for sleep recording sessions. The weight of the system (cable, swivel and the freely turning bar) is balanced so as to permit the rat to sleep in a natural position. Pre-amplifier in the upper right-hand corner. In the background tubes for the cooling system of the box for maintaining constant ambient temperature.

Figure 6: (right)

Sleep recording chamber. Rat with a transmitter fastened to its head, connected to chronically implanted EEG and EMG electrodes. Two rats are recorded simultaneously in two separate units. A recording unit consists of the plexiglass cage placed on a force recorder for registration of motor activity, and of two mechano-electrical balances for measurement of food and water consumption. One of the three antennae for receiving the transmitter signal is in the upper right-hand corner.

Figure 5:

Figure 6:



Pre-amplifier

The EEG and EMG signals were preamplified in the recording chamber. The pre-amplifier consisted of two completely separated, optically isolated amplifiers, one for EEG and one for EMG. Each amplifier was fed by two rechargeable accumulators with 100 guaranteed working hours. The input signal was amplified by a factor of 1000.

Polygraph

The pre-amplified EEG and EMG signals were passed through the power amplifier and plotted on paper by a dynograph (Offner, Beckmann), calibrated to 11 μV/0.5 cm. The usual paper speed was 3 mm/s.

Calibrator

The sine-wave calibrator had two independent outputs: 100 μV pp and 300 μV pp; (frequency 10 Hz). With these two signals the EEG and EMG channels could be tested alternatively. This device was very helpful to detect the source of artefacts. By exchanging the rat with the calibrator, artifacts caused by the animal itself or by the recording system could be identified. Furthermore the calibrator was used to define the amplitude of the rat EEG.

Data analysis

The vigilance states waking (W), non-REM sleep (NREMS) and REM sleep (REMS) or paradoxical sleep were scored for successive 12-s epochs by visual inspection of the polygraph records. The discrimination of the 3 vigilance states was made according to the frequency and amplitude of the EMG and EEG. EMG frequency and amplitude were high during W and low during NREMS and REMS. The EEG during W and REMS was characterized by low amplitude and high frequency waves, whereas during NREMS it showed typical high amplitude slow waves. During REMS the electroencephalogram showed a regular theta rhythm originating in the hippocampus.

States lasting less than 6 s were disregarded.
The EEG was often also recorded on analog magnetic tape (Instrumentation recorder, 3968 A, Hewlett Packard). After the experiment the tape was played back at four times the recording speed (r.s.), filtered (100 Hz low-pass corresponding to 25 Hz r.s.), AD converted (conversion rate 256 Hz corresponding to 64 Hz r.s.) and subjected to Fourier analysis on a PDP-11/20 computer using a Fast Fourier Transformation routine (epochs of 4 s). The spectral power of the following bands: 1.25 - 4.00 Hz, 6.25 - 9.00 Hz and 12.25 - 15.00 Hz was computed and plotted for consecutive 1 min periods (e.g. Figs. 25 and 31). For numerical analysis the power of the delta band was computed separately for the control period and for the period during or following drug application, and expressed relative to NREMS (total power/ % NREMS).

2. Telemetry

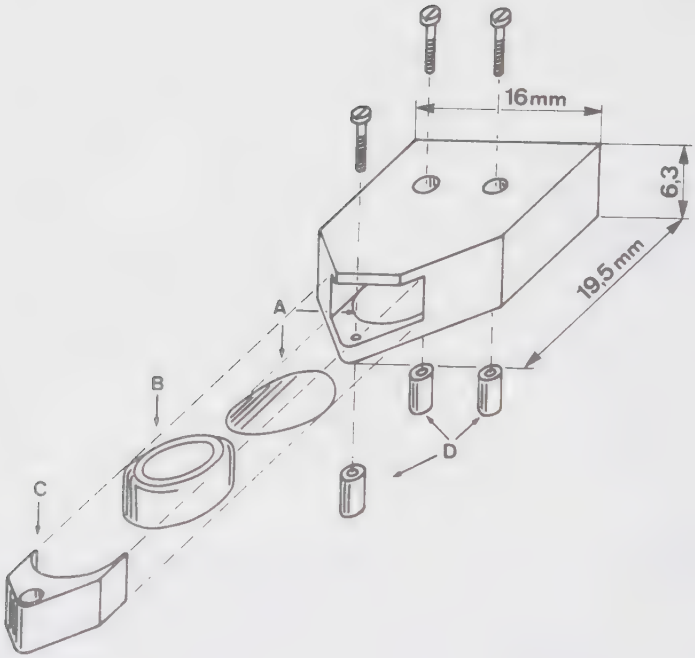
Operation

The implantation of electrodes was carried out as for cable recordings. The brain and muscle electrodes were soldered to 3 hollow gold-plated brass cylinders which were cemented to the skull at predetermined positions. The transmitter was fastened to these cylinders with screws which had the dual function of providing electrical contact and mechanical fixation. Gold wire electrodes (p. 16) were used to record the cortical EEG (parietal cortex) and the neck muscle EMG, a cerebellar electrode serving as a common reference.
At least 5 days were allowed for recovery before recordings were started.

Transmitter (Ruedin et al., 1978)

EEG and EMG were recorded with a miniature battery-powered, 2-channel FM-AM transmitter (weight: 3.0 g; 19.5 x 16 x 6.3 mm) on the head of the animals. (Fig. 7).

Figure 7:



Dimensional drawing of transmitter with screws and cylinders (D) for fixation on the skull, battery (B) and its contact Figure from P. Ruedin et al., 1978.

Experimental conditions

During the experiments the animals were kept in a sound attenuated separate room, with an ambient temperature of 22°C controled to within ± 0.3°C. They were kept individually (p. 20) in the cages they had been adapted to prior to the experiments.

Recording

The transmitter signals were picked up by 3 antennae, demodulated and fed into the state identification circuit (Neuhaus and Borbély, 1978). The following parameters were monitored throughout the experiments: the vigilance states W, NREMS and REMS; motor activity (p. 13) and food and water intake. Food and water intake were monitored by means of mechano-electrical balances which recorded the weight of the food and water containers (Fig. 6).

Data analysis

The automatic state identification system consisted of an online and of an offline stage. The online circuit determined at 10-s intervals whether the preset threshold values of the following integrated parameters were exceeded or not: activity (ACT); the ratio rectified EEG/ACT; theta activity (6 - 9 Hz); and the rectified EMG. All data for 23½ h per day were stored on paper-tape and read into a computer (off line stage). The vigilance states were determined for 10-s epochs and stored on disk together with the other behavioral parameters. The performance of the system was checked daily on the basis of polygraph recordings. The paper tape was changed daily usually between 09:30 and 10:00 and manipulations in the experimental room occurred at 3-day intervals within this time period. Data from these interruptions were not used for analysis.

E Drug application methods and effect of intraventricular infusion

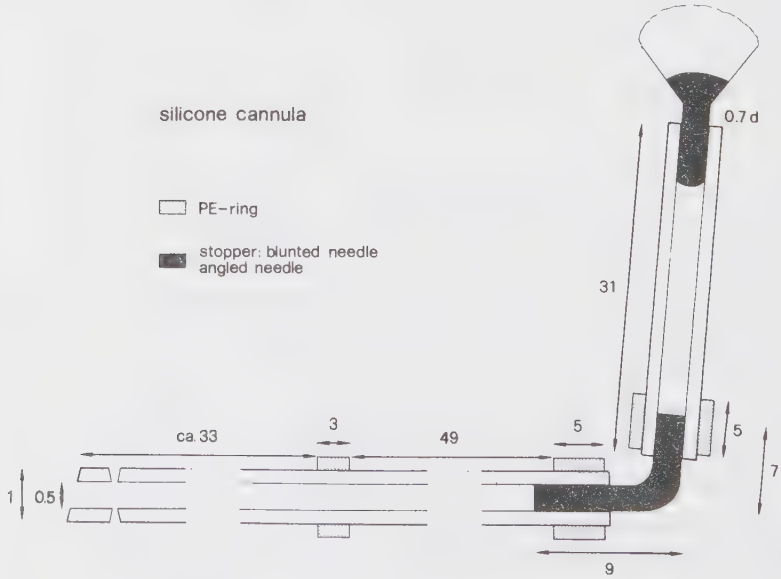
The route of administration of a drug alters the rate of absorption and consequently the duration of the effect. The nature of the solvent affects the rate of absorption.
The two main disadvantages of the classical methods for administering drugs to animals (e.g. intraperitoneal or intravenous injection) are that the entrance of the substance into the body is intermittent rather than continuous and that they imply handling of the animals with all the complications due to stress.
A substance may be more effective when it is administered by a slow and prolonged release rather than by repeated injections. Silastic capsules and other pellets implanted subcutaneously allow a relatively constant release of a drug over a long time period. Continuous infusions or perfusions may be performed by chronic catheters placed into a blood vessel or into the cerebral ventricles. A very slow drug infusion (1 µl/h) may be realized with a newer method, the osmotic minipump. An advantage of these methods lies in the fact that they are practicable in the undisturbed animal, a definite asset in sleep research.
The route of administration of a drug is important. Some of the problems to be considered are (i) unspecific effects in the periphery rather than the desired central effects; (ii) the ability of the drug to cross the blood-brain barrier or blood-CSF barriers; (iii) the distribution in the brain after systemic administration; (iv) concentrations prevailing at the target areas where a drug may produce either stimulation or block depending on the concentration; and finally (v) the actions on more than one area which may be mutually antagonistic or synergistic. Thus even the intraventricular route of administration may be too unspecific for some problems involving drug action in distinct brain areas. Nevertheless in our studies, the cerebroventricular infusion method has proved useful for

studying sleep in the rat. We have not yet used further refinements of the technique which may involve infusing very small volumes into discrete regions of the brain and thus stimulating chemically circumscribed cell groups.
In our studies we varied localization of the intraventricular infusion cannula to exert a preferential influence on various parts of the ventricular system. In some studies we chose the third ventricle as a site of injection because of its proximity to the hypothalamus. The brainstem can be most immediately affected by infusions into the fourth ventricle. Unfortunately, in our hands infusions into the 4th ventricle were accompanied by too many irregularities to be yet of any practical usefulness.

1. Drug administration

Cannula

Figure 9:



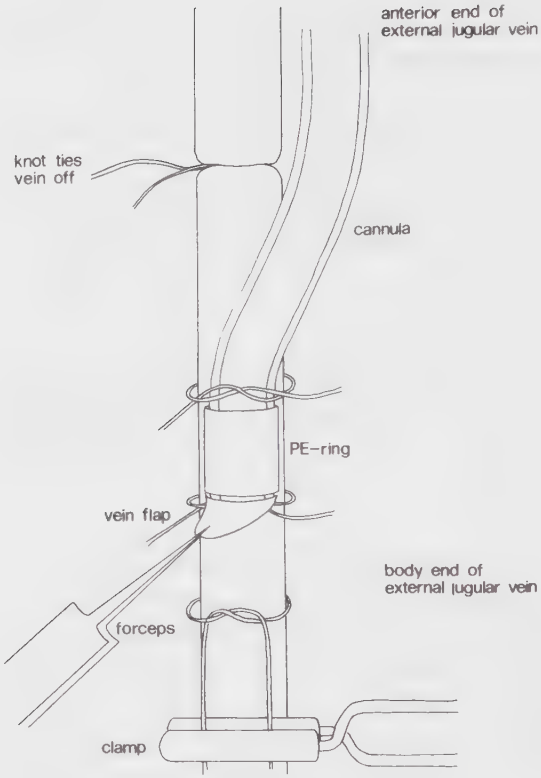
All measures are given in mm.

Pilot studies with polyethylene (PE) tubing showed that PE is not tolerated very well; therefore all experiments were carried out with silicone tubing (Silastic medical grade tubing, Dow Corning, 0.5 mm id 1.0 mm d). A slightly slanted cannula tip and two small slits in the wall on opposite sides at a distance of 3 mm from the inner end prevented coagulation. A PE ring was fixed ca 33 - 40 mm from the tip. Prior to surgery the catheter was filled with saline and closed at the outer end with a metal plug (a shortened and blunted syringe needle, 0.7 mm d). After surgery saline was replaced by a 10 : 1 saline-heparin solution (500 units/ml).
Some catheters were filled with a more viscous solution of saline and PVP (polyvinylpyrrolidone). In our experiments no advantages were seen as compared to a filling of heparine/ saline solution.

Surgery

Under barbiturate anesthesia the rat was placed on its back with the forelegs fixed on a platform. According to the method described by Steffens (1969) a longitudinal incision of ca 15 mm was made in the skin between the sternum and the right foreleg. The external jugular vein was prepared just anterior to the junction with the anterior jugular vein and tied off at the head end. Fig. 10. In order to avoid leakage of blood or aspiration of air into the circulation, a silk thread was run under the vein as near to the sternum as possible, tied loosely and weighed with a clamp. Thus it could be quickly tied when the cannula had been inserted. A very small incision was made in the vein near the head end pointing toward the body. The flap of the cut vein was picked up with a very fine pointed forceps, and the filled cannula was carefully inserted, taking care not to puncture the vein with the pointed tip of the tubing. The weight on the vein was removed and with circling movements the catheter was teased further into the vein up to the PE ring. The tip of the catheter was thus approximately

Figure 10:



Insertion of cannula into the vein

situated at the entrance of the right atrium. Four ligatures, two above and two below the ring anchored the cannula to the muscles and fixed the catheter in this position. It was important that these ligatures be placed carefully, since the tip of the cannula could become occluded later if the endothelium was irritated by cannular movements within the vein. The catheter plug was then removed, and, if the catheter was placed correctly, either some of the heparine/saline solution was aspirated or the tubing filled with blood. A large hypodermic needle was inserted subcutaneously from a slit in the skin on the skull,

behind the ear to the incision in the breast. The catheter end was inserted into the needle, and by pulling back the needle placed under the skin. The length of the tubing was corrected so that it was not too taut. Before fixing the catheter to the skull, the tip of the tube was fixed to an angled hypodermic needle which was cemented to the skull. To assure a tight fit, the tube was fixed to the metal with a tightly fitting PE ring. (Fig. 9).
All metallic ends were polished smooth.
The animals recovered rapidly from this operation. To avoid obstruction of the catheter, it was filled daily with a new heparine/saline solution, thus remaining patent for approx. 3 weeks.

Infusions

For infusions the cannula stopper was removed and replaced by a small Y-shaped metal tube (two syringe needles welded together). PE-tubing was attached to the free ends of the Y, one side filled with saline and the other with a drug solution. Metal and PE-tubing were loosely connected to the EEG cables, to the balanced beam, and led out of the recording chamber to the infusion pump (Precidor 3000, Infors AG Basel). Usually the saline tubing was attached to the pump and saline was infused for 1 - 2 h before it was replaced with the drug tubing for 40 - 100 s, infusion rate approx. 100 µl/min. In this way drugs were applied without disturbing the sleeping animal and without interrupting the infusion flow.

Electrocardiogram (ECG)

Two isolated copper or stainless steel wires were inserted under the skin behind the arms of the animal. The wire tips were bent to form a spiral (ca 0.7 mm d) which was fixed to the skin with two small stitches. The other end of the wires was soldered to the plug next to the EEG electrodes and cemented to the skull.

The ECG signal was amplified with a strain gage coupler (Offner, Beckmann) and plotted by a Beckmann RP Dynograph. Heart pulses per minute were counted visually.

Infusion, sleep and ECG

EEG and ECG were measured in four rats before and during a 1 hour infusion of saline into the jugular vein (infusion rate 100 µl/min). Infusion in the sleeping animal had no influence on the rate of heart beat.

2. Chronic release from implanted pellets

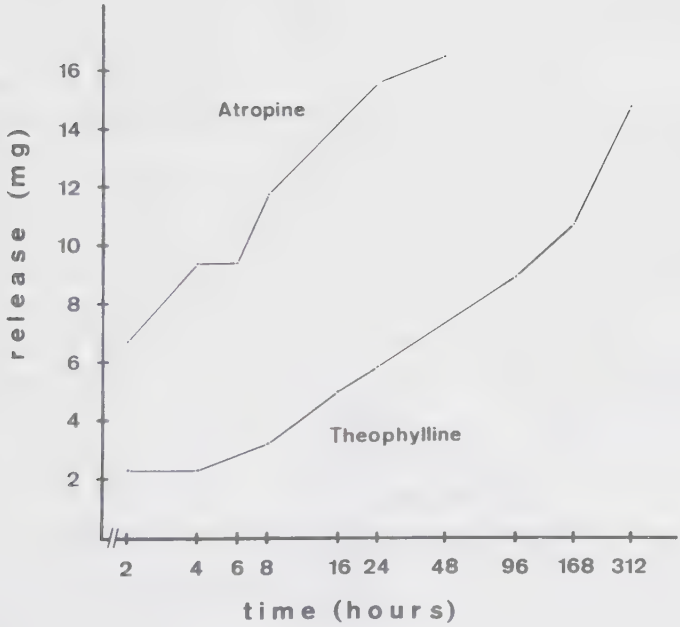
Disk shaped pellets (8 mm d, 2 mm thick) were pressed (3000 kp/cm^2 hydraulic pressure, Apex hydraulic press) containing 30 mg atropine sulphate with 70 mg glycerinetripalmitate, or 50 mg theophylline with 100 mg glycerinetripalmitate. Under brief ether anesthesia the pellets were implanted subcutaneously under the skin of the back and removed after various time intervals.

Release rate of subcutaneously implanted pellets

Two pellets, each containing either 30 mg atropine sulphate (6 rats) or 50 mg theophylline (8 rats), were implanted subcutaneously. The release rate of atropine and theophylline was determined by titration with NaOH/0.1 m for 48 and 312 hours, respectively. (For titration the pellet was dissolved in 10 ml hot water and 4 ml $AgNO_3$ 0.1 n).
Figure 11 shows the rate of release of the two compounds. A high release within the first 2 h is followed by a relatively constant release rate (note the logarithmic time-scale). A decrease in the release rate of atropine sulphate between 24 and 48 h is possibly due to a tissue reaction that was seen with this compound.
Pilot studies comparing control pellets with PVP (Kollidon) and Mg-stearate or PVP alone or glycerinetripalmitate showed best

tolerance with the latter fat as carrier.

Figure 11:



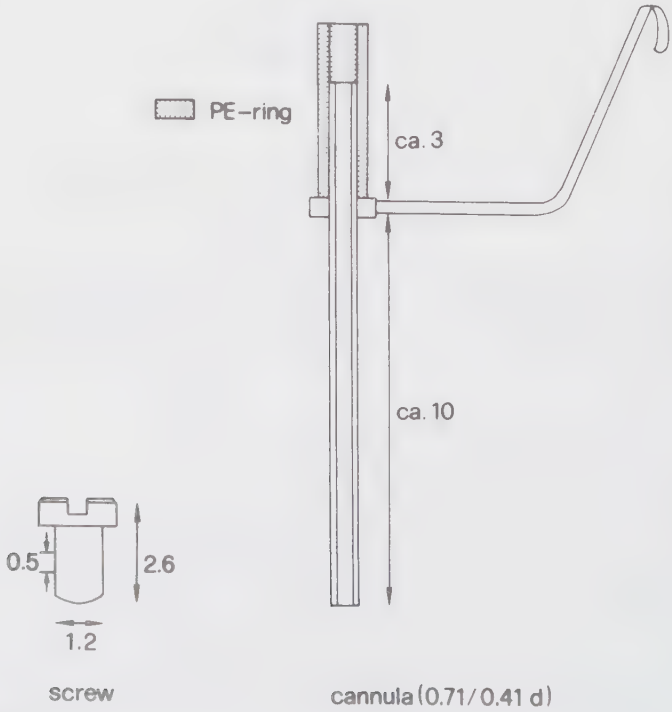
Release rate of subcutaneously implanted pellets containing atropine sulphate (30 mg) or theophylline (50 mg). Each point represents the arithmetic mean value of at least two determinations.

3. Cerebroventricular drug administration

The implantation was performed under pentobarbital anesthesia. The skull of the animal was immobilized with a headholder, and after exposing the skull, a 22 gauge stainless steel guide cannula (Fig. 8) was inserted through a drill hole into the lateral, third or fourth ventricle. For implantation into the lateral and third ventricle the cannula was advanced perpendicularly at the coordinates AP 5.4, L 1.7, D 2.2 - 3.5 (Pelle-

grino and Cushmann, 1967) for the lateral and AP 3.5, H 3.8 - 4.4, L 0.0, (König and Klippel, 1963) for the third ventricle. It is essential to stay at a safe distance from the blood sinuses situated directly under the skull in order to avoid hemorrhage. For this reason a large hole (2.3 mm) was drilled for the third ventricle cannula to allow a gentle displacement of the blood vessel with a needle, before inserting the cannula into the brain.
To avoid perforating the blood sinuses during implantation into the rostral part of the 4th ventricle, the cannula was advanced at an angle of 35° relative to the interaural axis with the coordinates AP - 0.75, L 3.5, D 5.0 - 6.0.

Figure 8:



All measures are given in mm.

In some animals the infusion pressure was recorded on a Beckmann Offner type 700 strain gauge coupler (type 9872) during the insertion of the cannula into the brain.
Artificial cerebrospinal fluid (aCSF) was continuously infused (3 - 10 µl/min) until a sharp drop in pressure indicated the correct location of the cannula tip in the ventricle cavity. Fig. 12 (Goodrich et al., 1969).
In later experiments the correct placement of the cannula was verified by the gravity inflow method: for surgery the inflow cannula was inserted into the guide cannula and connected to the infusion tubing filled with aCSF. During insertion into the brain, before fixing the cannula to the skull, the tubing was elevated above the level of the rat's head and penetration of the ventricular lumen was verified by the abrupt gravity inflow of the perfusate. After fixation of the guide cannula, a 28 gauge stainless steel mandril was inserted which could be replaced by a 28 gauge inflow cannula for infusion experiments. Holding screws and dental cement were used to fasten the guide cannula to the skull.

Intraventricular administration procedure

For intraventricular injection the animals were held manually and a total volume of 1 - 3 µl fluid was injected within 1 - 2 min. To avoid backflow the infusion cannula was left in place for another minute before the rat was returned to the recording chamber. Usually, intraventricular infusions were administered to animals which had been previously adapted to sleep with the tubing connected to their head. At the beginning of each experimental session, the mandril was replaced by the injection cannula which was connected via a polyethylene tubing to the infusion pump (Precidor, Type 5003) outside the recording chamber. Thus handling of the animal at the time of drug administration was avoided.
The infusion rate was usually 3.0 - 3.3 µl/min and in some experiments 0.3 and 1 µl/min. An infusion rate of 3.3 µl/min is approx. equivalent to the rate of production of natural CSF

in the adult rat (Cserr, 1965; Bass and Lundborg, 1973). In several animals the intraventricular pressure was monitored during repeated 60 min infusion periods at an infusion rate of 3.3 µl/min and no increase in pressure was found if the cannula was correctly located in the ventricular lumen (Goodrich et al., 1969).

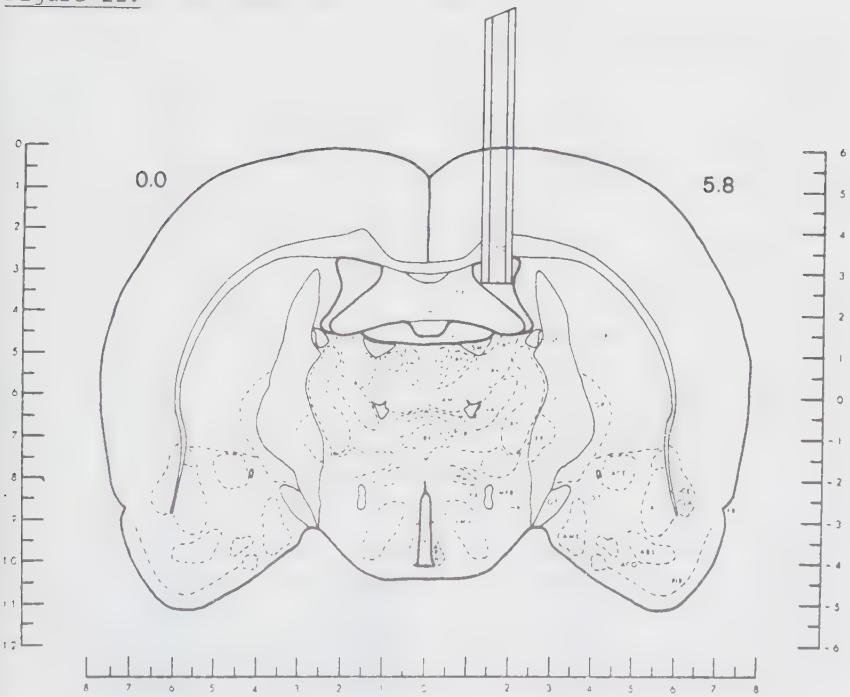
Histology

At the end of each experiment with intraventricular infusions, 3 µl trypanblue (0.5g/100 µl H_2O; acid dye) was injected into the ventricle of the conscious rat. After 30 min the animal was decapitated, the brain removed and stored at -20°C for at least 24 h. The brains were sliced on a cryostat into 15 µm sections and collected on slides which had previously been immersed in a gelatin solution (0.9g/100 ml H_2O) and dried. Some sections were left to dry for a few days before they were dipped for 10 min in Kernechtred (1g/l $Al_2(SO_4)_3$ 5%; basic dye), rinsed in distilled water and dehydrated (ethanol 70% - 100% and xylol). The colored slices were covered with Etellan.

Cerebroventricular infusion and motor activity

Although the cerebroventricular administration of chemical substances is widely used in neuropharmacology, the physiological and behavioral consequences of the infusion procedure *per se* have received little attention. Fencl and coworkers (1971) have reported that intraventricular infusion of aCSF, natural CSF from normal goats and physiological saline in rats causes a depression of motor activity for 6 h of the dark phase immediately following the infusion. (Infusion rate 3.3 µl/min). We attempted to clarify this issue with the present experiments in which we studied the influence of varying infusion rates and volumes on motor activity of the rat.
Motor activity recordings were obtained from 6 rats implanted with a chronical cannula in the lateral ventricle (p. 13).

Figure 12:



Brain section from Pellegrino and Cushman (1967) indicating placement of the chronic guide cannula in the lateral ventricle.

Activity was recorded for 23 h periods for two control days without infusion (one before and one after the infusion days), one sham infusion day, and following 5 different infusions (p. 33). Each animal received 4 infusions at intervals of at least 48 h. The following rates and durations of infusion were used: aCSF II (Table 1): 1 µl/min-15 min (aCSF 1-15', 1 µl/min-45 min (aCSF 1-45'), 3 µl/min-15 min (aCSF 3-15'): CSF (p. 57): 3 µl/min-15 min (CSF 3-15'). The order of the aCSF infusions varied, while CSF was administered last. For sham infusions (5 rats) the cannula was inserted and left in place for 15 min without turning on the pump. The end of each infusion period

was made to coincide with the onset of the dark-phase when the recordings were started.

Table 1

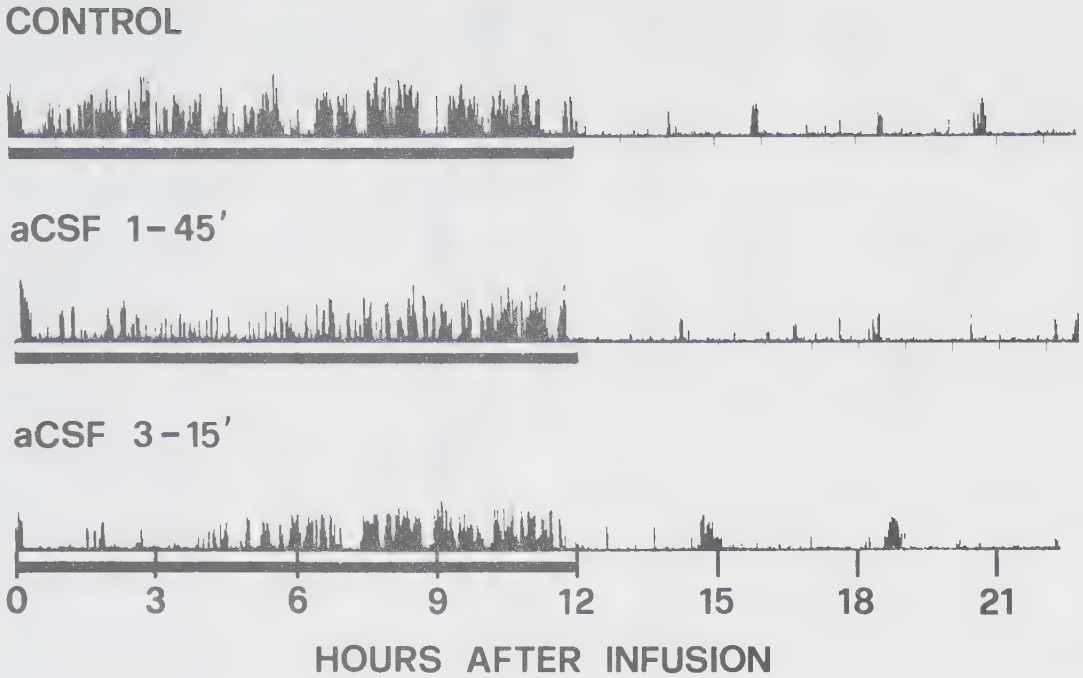
Concentration of ions (mM) in 3 types of artificial cerebrospinal fluid (aCSF)

	aCSF-I	aCSF-II	aCSF-III
Na^+	150.0	127.6	127.6
Ca^{2+}	1.8	1.3	1.3
K^+	5.1	2.5	2.5
Mg^{2+}	-	2.0	1.0
Cl^-	158.7	136.7	134.7

A prolonged depression of motor activity was seen after infusion of aCSF 3-15' and 1-45', and CSF 3-15' (Figs. 13 and 14). The original records (Fig. 13) illustrate the attenuation and shortening of the activity bouts and their gradual recovery. The histograms based on the mean hourly values show the prolonged decrease in activity which occurred after rapid and short infusions as well as after slow and long infusions. However, because of the fluctuation of motor activity within a recording period, and the variability between animals, only some of the hourly values were significantly below the control level (Fig. 14 asterisks). By taking longer periods as units for analysis, the significant decrease of activity becomes more clearly evident (Fig. 14 horizontal bars). Activity during the subsequent light-phase did not show significant differences (data not shown). Following infusions of aCSF 1-15' and sham infusions, the depression of activity was limited to the first 2-3 h.

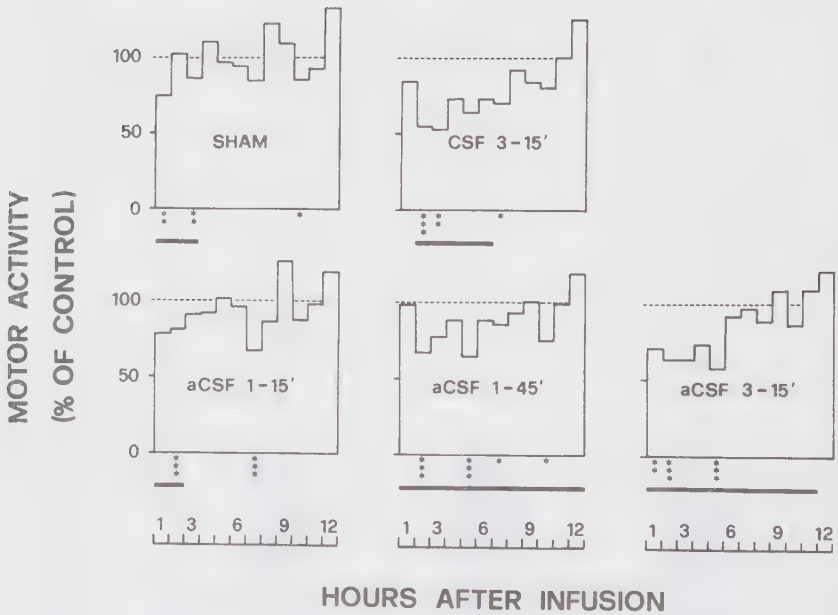
In comparison with the control days (+4.2g) and the sham infusion days (+4.6g), the mean daily gain in body weight was reduced after the infusion days (aCSF 1-15' : + 0.3 g ($p < 0.025$);

Figure 13:



Effect of cerebroventricular infusion on motor activity pattern. The original plots show for the same animal records obtained without infusion (control), after infusion of artificial CSF at a rate of 1 µl/min for 45 min (aCSF 1-45') and at a rate of 3 µl/min for 15 min (aCSF 3-15'). The bar beneath the records indicates the dark phase. Motor activity is plotted in arbitrary units.

Figure 14:



Effect of cerebroventricular infusion on motor activity. Histograms show mean hourly activity values for the 12-h post-infusion period (dark phase) expressed as percentage of control. The mean hourly values of 2 control days were defined as 100 % (dashed line). The histograms show the effect of a sham infusion; infusion of natural CSF at 3 µl/min for 15 min (CSF 3-15'); artificial CSF at 1 µl/min for 15 min (aCSF 1-15') or 45 min (aCSF 1-45'), and at 3 µl/min for 15 min (aCSF 3-15'). Asterisks below histograms indicate hourly values that were significantly below control. Bars below histograms denote the length of a period starting at hour 1 or 2 for which activity was below the $p = 0.025$ level (one-sided paired t-test; *$p < 0.05$; ** $p < 0.025$; *** $p < 0.01$).

1-45': +0.7 g (ns); 3-15': +2.5 g (ns); CSF 3-15': -3.3g ($p < 0.05$; one-sided paired t-test, comparison with control). A compensatory increase exceeding the control values was invariably seen on the second post-infusion day.

Our results are consistent with the observations by Pappenheimer and colleagues (Fencl et al., 1971) , who reported that infusions of aCSF or CSF depress motor activity during the 6-h post-infusion period. However, the present experiments do not support the authors' interpretation that the reduction of activity represents a compensation of the sleep loss incurred during the infusion procedure. In our hands, sham infusions caused only a short-lasting, immediate depression of activity, whereas both rapid and short infusions, and slow and long infusions, caused a prolonged and often delayed depression. A difference between sham and real infusions was apparent also from the decreased weight gain or weight loss after the infusion days.

What were the factors responsible for the changes observed in the post-infusion period? The infusion rate of 3 µl/min, even though being higher than the estimated secretion rate of natural CSF (2.2 µl/min; Cserr, 1965), cannot account either for the depression of activity which was observed also after a 45-min infusion at a rate of only 1 µl/min. The total volume of the infusate may be more important, since the long-lasting depression of activity after infusion of 45 µl aCSF was not seen after infusion of only 15 µl aCSF. The difference in the duration of the effect after aCSF 3-15' and natural CSF 3-15' may indicate that the ionic composition of the aCSF used may have differed from that of natural CSF. On the other hand, it is tempting to interpret the different effect of natural CSF as being due to the presence of sleep and activity-modifying factors (Sachs et al., 1976). One may even consider the possibility that a certain volume of fluid infused into the ventricular system might lead to the release of endogenous sleep-promoting substances such as factor S or arginine vasotocin which

in minute amounts are reportedly capable of reducing motor activity (Fencl et al., 1971; Krueger et al., 1978) or REMS (Pavel et al., 1977) for several hours. These speculations should not distract from the fact that the mechanisms of action underlying the present results remain elusive.
It is evident that the effects of intraventricular infusions may not be adequately interpreted by looking only at differences in motor activity. This consideration prompted the next series of experiments.

Cerebroventricular infusion and sleep

Feasibility

With the present experiments we intended to evaluate the feasibility of administering intraventricular infusions to sleeping animals and the usefulness of this technique for sleep research.
Three aspects have to be considered: first, does an intraventricular infusion of aCSF per se have an arousing effect on the sleeping rat?; second, considering the results from experiments on cerebroventricular infusion and motor activity, what are the after-effects of an infusion on sleep?; and third, does intraventricular infusion influence the distribution of the vigilance states during the infusion period itself? The latter is an important problem, if the animal is to be infused for a longer period of time with a submaximal drug dose so as to avoid an arousal effect of a small volume with a high drug concentration.

Ca-ions

Half a century ago Demole (1927) and Cloetta & Fischer (1930) demonstrated that the intracerebral injection of a calcium chloride solution induced a sleep-like state in the cat and in the rabbit. Localized perfusion experiments in cats confirmed these findings (Veale & Myers, 1971). Studies concerned with Ca effects on the rat centered on food-intake and body temperature

(Myers et al., 1972; Myers and Brophy, 1972; Myers and Bender, 1973; Myers and Melchior, 1976; Gisolfi et al., 1976). We were interested to evaluate a possible sleep inducing effect of intracerebral Ca infusion in this species.

Mg-ions

The four ions directly involved in the excitability of the nervous system, can be divided into two groups: stimulants, Na and K, and depressants, Ca and Mg. Since little work has been published on the effect of intraventricular Mg application in relation to its depressant effects, we tested the effect of Mg-ions on sleep. Seoane and Baile (1973) reported dose dependent feeding responses after cerebroventricular injections of Ca or Mg in sheep. In our laboratory (Toszhegi et al., 1978) Ca infusions were shown to elicit immediate and prolonged feeding in rats, results that prompted the investigation of Mg-ions.

Experiments with artificial cerebrospinal fluid (aCSF)

The effect on the sleep states of three different types of artificial cerebrospinal fluid (aCSF) infused into the lateral ventricle of chronically implanted rats were studied (pp.31 and 33). The ionic composition of aCSF was based on infusates used by Myers in previous studies (Myers et al., 1972; Myers, 1972). The experiments with aCSF-II and aCSF-III were prompted by the observation that infusions with aCSF-I led to marked alterations of the sleep states (Table 2).

The effects of excess Ca or Mg were investigated by raising the Ca-level in the aCSF by a factor of 2-3. The pH of the infused CSF was 5.9 - 6.5. No buffer was added.

Pilot studies were carried out with aCSF-II containing 49.99 mM NaCl or aCSF-I with a NaCl concentration equivalent to aCSF-I-3x CaCl (NaCl: 157.18 mM), to compensate for total molarity differences of the infusates. These variations of the NaCl concentration in the infusate had no effect on sleep as compared to aCSF-I or -II.

Table 2: Sleep parameters during infusions into the lateral ventricle. Mean values with standard error in parenthesis for the control condition without infusion, for infusion with artificial CSF (aCSF) and with excess calcium (Ca-x2-3 and Ca-x3). Three types of aCSF were used (see Table 1). W: waking, NREMS: nonREM-sleep, REMS: REM-sleep. Asterisks indicate the significance level in comparison with the corresponding aCSF-value (one-sided paired t-test): * $p < 0.05$; ** $p < 0.025$; *** $p < 0.01$; n = number of animals.

Experimental condition	Infusion rate (µl/min)	Distribution of vigilance states (percent of recording time)			REMS per total sleep (percent)	n
		W	NREMS	REMS		
Experiment 1						
uninfused control	-	13.4 (3.1)	72.3** (3.7)	14.3*** (1.5)	16.7*** (1.9)	7
aCSF-I	3	12.8 (1.1)	80.4 (1.5)	6.8 (1.2)	7.7 (1.3)	7
aCSF-I+Ca-x2-3	3	12.0 (1.8)	75.2* (2.3)	12.8* (2.4)	14.0* (2.7)	8
Experiment 2						
uninfused control	-	14.9 (0.9)	68.8*** (1.2)	16.3 (0.9)	19.1* (1.0)	9
aCSF-II	3	12.4 (1.8)	73.8 (1.9)	13.8 (1.6)	15.8 (1.8)	7
aCSF-II+Ca-x3	3	12.3 (2.2)	67.4** (2.6)	20.4** (2.1)	23.2*** (2.3)	6
aCSF-III	3	13.9 (2.6)	70.8 (1.4)	15.3 (1.6)	17.6 (1.5)	6
uninfused control	-	17.6 (1.4)	67.7 (0.8)	14.7 (0.9)	20.6 (0.4)	5
aCSF-II	1	18.0 (1.3)	68.6 (1.2)	13.4 (1.8)	18.2 (2.5)	5
aCSF-II+Ca-x3	1	18.6 (2.2)	67.8 (1.9)	13.6 (0.6)	20.0 (0.8)	4

aCSF-I and excess Ca

After the animals had been habituated to the recording leads, the EEG and EMG were recorded for approximately 60 min to obtain a baseline for the distribution of the sleep states (Table 2: uninfused control). In a second recording session on a subsequent day, aCSF-I was infused for 65 - 115 min (mean 82.6 min) into the lateral ventricle.
Finally, in a third session, usually on the day after aCSF-infusion, aCSF-I containing 2-3 times the usual concentrations of calcium (Ca-x2-3 ; 3.6 - 5.4 mM) was administered for 60 - 93 min (mean 71.9 min). In all experiments infusion was started when the animal had been asleep for at least 30 min. Sleep was never disrupted by the onset of infusion. In one aCSF-session a prolonged period of waking occurred 46 min after infusion onset, and in two Ca-x2-3-sessions waking and feeding were observed at a latency of 38 and 44 min. In these experiments, only the periods prior to waking were used for the computations. It can be seen from Table 2 that the percentage of waking was similar under all recording conditions, whereas marked differences occurred between the percentages of NREMS and REMS. In comparison to the control session, REMS was reduced by more than 50 % during aCSF-I-infusion. The reduction of REMS was mainly due to a shortening of REMS-episodes, since the percentage of episodes exceeding 2 min was reduced from 48.0 to 25.0. In contrast to aCSF-I-infusions, the percentages of REMS and NREMS during infusion of Ca-x2-3 were not significantly different from the uninfused control values. The Ca-x2-3 values for REMS/TS and the percentage of REMS-episodes exceeding 2 min (40.3 %) were only slightly lower than the control values. Four animals infused with Ca-x5 woke up and started feeding at latencies ranging from 16 to 42 min (mean 24 min). Considering equivalent time periods in control sessions without infusion and with aCSF-I, the following values for REMS/TS were obtained: Uninfused control 18.8 % *** (1.8 SEM); aCSF-I: 5.8 % (1.7 SEM); Ca-x5: 19.2 % ** (3.0 SEM) asterisks indicate significance level

in comparison with aCSF-I as in Table 2). Thus increasing the Ca-concentration in the infusate by a factor of 5 yielded REMS/TS values that were significantly higher than those for aCSF-I, and even slightly higher than the control level.

aCSF-II and aCSF-III

Table 2, Experiment 2. The general procedure described for experiment 1 also applied to the present experiments. The infusions usually lasted 60 min, unless a prolonged period of waking occurred. In this case, only the period prior to waking was used for computation. The control and experimental values for individual animals were obtained from repeated 60-min recordings.

In contrast to the experiments with aCSF-I, infusions of aCSF-II and aCSF-III at a rate of 3 µl/min caused only a minor reduction of REMS and REMS/TS (Table 2). The values of REMS and REMS/TS were significantly higher for infusion with excess Ca than for aCSF alone (comparison with aCSF-II: see Table 2 for significance level; comparison with aCSF-III: REMS, $p < 0.025$; REMS/TS, $p < 0.05$), and even exceeded the control level obtained without infusion. The differences between aCSF-II and aCSF-III were not significant.

The results obtained with a reduced infusion rate (1 µl/min) are shown in the bottom part of Table 2. Infusion of aCSF-II caused only a slight, non-significant reduction of REMS and REMS/TS, whereas REMS/TS was close to the control level during infusion of excess Ca.

aCSF-II and excess Mg

Sleep was recorded in an additional group of 6 rats which were infused with aCSF-II as control (1 µl/min) and with aCSF-II containing 3 times the usual concentration of Mg (Mg-3x; 6.0 mM). No significant difference was found in any of the sleep parameters during infusion of aCSF-Mg-3x as compared to aCSF-II. (W: 12.8 ± 2.3 ; NREMS: 68.8 ± 2.6 ; REMS: 18.3 ± 2.0 ; REMS/TS: 21.0 ± 2.2).

To test the hypothesis that high concentrations of Mg may elicit feeding, rats were observed during the 30 min infusion into the lateral ventricle (3 µl/min) of aCSF-III (control) and aCSF-III with excess Mg (10, 15, 20 and 30 mM), and for 30 min after the infusion. The occurrence of feeding and other behaviors within 30-sec epochs was scored for 5-min bins (n = 6-8) and expressed as a percentage value (ten 30-sec epochs with feeding = 100 %). Fig. 15, Table 3.

Table 3

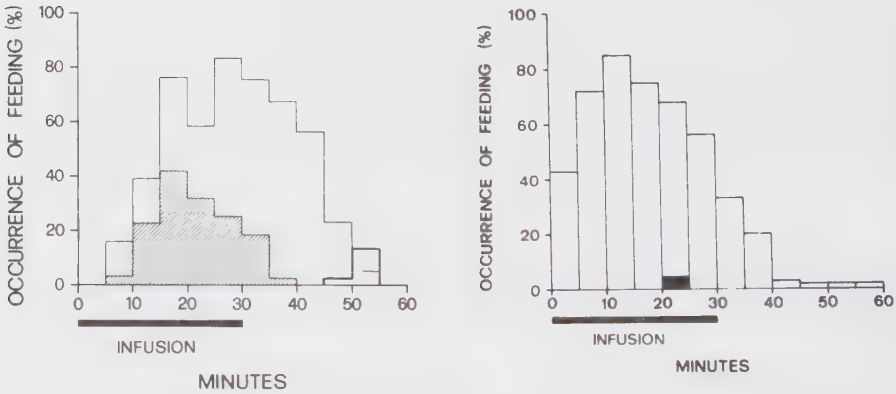
Occurrence of feeding (%) during and after 30 min infusion into the lateral ventricle of aCSF-III (Mg 1 mM) and aCSF with excess Mg (10, 15, 20 or 30 mM).
Numbers represent arithmetic mean values of 6-8 experiments.
Dashes indicate no feeding.
The occurrence of feeding within successive 30-s epochs was scored for each 5-min bin and expressed as a percentage value (ten 30-s epochs with feeding = 100 %).

	Infusion											
Time:min / Mg mM	5	10	15	20	25	30	35	40	45	50	55	60
1	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	8.6	14.3	-	-	-	-
15	-	3.1	22.5	41.9	31.9	25.0	18.1	2.5	-	-	-	-
20	-	9.2	33.3	41.7	60.8	72.5	34.2	24.2	2.5	-	-	-
30	-	16.0	39.0	76.0	58.0	83.0	75.0	67.0	56.0	23.0	5.0	-

Infusion of Mg (15, 20 and 30 mM) into the lateral ventricle of awake rats invariably induced vigorous and prolonged feeding a few minutes after infusion was started. The duration of the effect was proportional to the concentration of excess Mg. 30 mM Mg evoked feeding for 25 min after the end of the infusion. No other behaviors like wet dog shakes or ataxia that were present after excess Ca-infusions (Tószeghi et al., 1978)

were seen with excess Mg. No feeding was elicited with Mg-infusions into the fourth ventricle even at the highest concentration that was used for lateral ventricle infusions; these observations correspond to the negative results reported for Ca (Tószeghi et al., 1978).

Figure 15:



Occurrence of feeding (%) during and after 30 min infusion into the lateral ventricle, of left figure: aCSF with excess Mg, 15 mM, (hatched bars), and 30 mM (white bars) and control aCSF-III Mg 1.0 mM (dark bars); right figure: (from Tószeghi et al., 1978) aCSF with excess Ca, 18.2 mM (white bars) and control aCSF-I Ca 1.82 mM (dark bars). Bars represent arithmetic mean values of 6 - 8 experiments. Horizontal bar below histogram indicates infusion period. The occurrence of feeding within successive 30-sec epochs was scored for each 5-min bin and expressed as a percentage value (ten 30-s epochs with feeding = 100 %).

Discussion

The present experiments have clearly demonstrated the possibility of administering intraventricular infusion to sleeping rats without disrupting sleep. The infusion technique allows the study of sleep while subjecting the 'inner surface' of the brain to subtle changes in the chemical environment.
It is also evident, that the effects of intraventricular infusions may not be adequately interpreted by purely behavioral criteria. Thus the marked shift in the distribution of the sleep states during aCSF-infusions would have passed unnoticed without EEG-recordings, since the distribution of sleep and waking was not altered. In analogy to the present results, a significant reduction of REMS occurred when an unspecified aCSF 'similar in composition to human CSF' was injected into various brainstem sites in the cat (Amatruda et al., 1975).
The massive reduction of REMS observed during infusion of aCSF-I was undoubtedly a consequence of the ionic composition of the infusate, since infusions with aCSF-II and aCSF-III caused only a minor decrease of REMS. The latter types of aCSF approximate relatively closely the ionic composition of natural CSF (Reed et al., 1967 ; Chutkow, 1968). Since a reduction of flow rate by a factor of 3 yielded a comparable distribution of the sleep states, we conclude that infusions administered for 1 h at 3.3 µl/min do not lead to a significant alteration of sleep.
During infusions of excess calcium the percentage of REMS was significantly higher than during infusion with aCSF. REMS was not only increased in absolute terms, but also when expressed as a percentage of total sleep (REMS/TS). Thus excess calcium induced a shift from NREMS to REMS. This result demonstrates that specific ionic changes in the CSF may selectively influence a sleep state. In accordance with this result Kripke et al. (1978) reported a relation of total plasma calcium with REMS and with stage 2 sleep in human subjects. Ca had a significant in-phase temporal association with REMS.

However, neither the mechanisms underlying the REMS-enhancing action of calcium nor those mediating the various physiological and behavioral effects of other ions, are known at the present time. It is possible that excess calcium may exert some of its actions by modifying the metabolism or release of transmitters. In fact, the perfusion of the hypothalamus with excess Ca has been shown to enhance the release of serotonin (Myers et al., 1970; Veale and Myers, 1971). Moreover, an activating effect of calcium on tryptophan hydroxylase has been recently described (Hamon et al., 1978). Since serotonin is a transmitter that has been implicated in the regulation of sleep, serotonergic mechanisms might mediate the effects of calcium observed in the present study. On the other hand, experiments with antagonists of various transmitters did not indicate that a single transmitter system is responsible for Ca-induced feeding (Myers et al., 1972; Myers and Bender, 1973).

Since calcium is involved in many different neurochemical processes, it is difficult to account for its behavioral effects in terms of specific mechanisms or sites of action. Yet, because of its ubiquitous occurrence, calcium may be ideally suited to assume important regulatory functions. Thus Myers and coworkers have proposed the setpoint of core temperature is regulated by the Ca/Na ratio in the hypothalamus (e.g. Myers and Brophy, 1972).

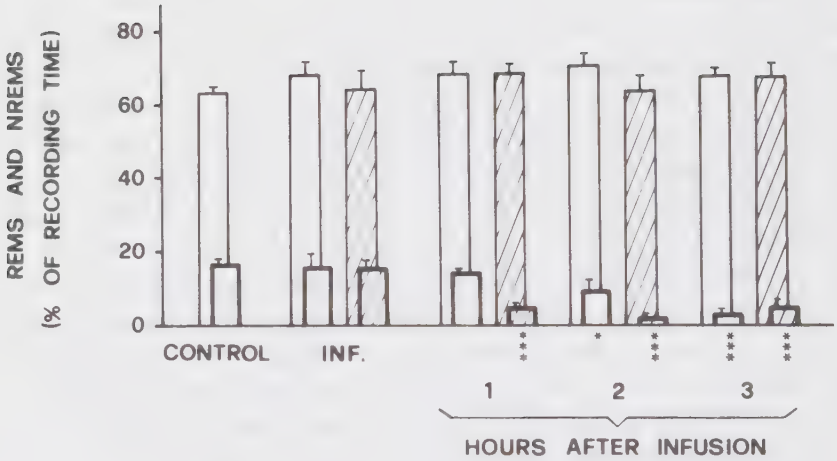
A 30-min infusion of excess magnesium (20-30 mM) in the lateral ventricle induced reliably feeding in the rat, comparable to results obtained in sheep (Seoane and Baile, 1973), whereas a rapid infusion of excess Mg had been little effective in a previous study (Myers and Bender, 1973). This indicates that an altered steady state in the ventricular milieu underlies the behavioral effects induced by the ventricular administration of ions.

Post-infusion effects

Sleep was recorded in the 12-h light-phase of 6 rats during and after infusion of aCSF-II or CSF at a rate of 3 µl/min for approx. 20 min, and compared with control recordings without infusion (5-7 h per animal). The vigilance states W, NREMS and REMS were scored (p. 21). Fig. 16 illustrates the effect of infusions on sleep. Although REMS was little changed during the infusion period (INF), it was significantly decreased after the infusion. The percentage of NREMS was not significantly altered. During the post-infusion hours, rapid successions of a high-amplitude slow-wave pattern and an electrographic arousal pattern without behavioral concomitants, were often seen.

The selective depression of REMS in the post-infusion period was unexpected since the sleep states had been little affected during a 60-min infusion period of aCSF in former experiments (p. 42). Since REMS remained close to the control level also during the infusion periods of the present experiments, the subsequent depression must be regarded as a delayed reaction to the infusion or to its discontinuation. It may not be coincidental that the two parameters most affected during and after infusion were motor activity and REMS, because common generating mechanisms for active waking behaviors and 'active sleep' have been proposed on the basis of electrophysiological and behavioral evidence (Borbély, 1978a, 1978b).

Figure 16:



Effect of cerebroventricular infusion of artificial CSF (light bars) and natural CSF (hatched bars) on REMS (REMS, low bars) and NREMS (high bars). Bars represent mean values and SEM (N = 6) obtained for control sessions without infusion (CONTROL), for the infusion period (INF.), and for the indicated hours after infusion. REMS and NREMS are expressed as percent of recording time. Asterisks indicate significant differences of REMS from control. Differences between the REMS-values for aCSF and CSF were significant for hour 1 and 2 ($p < 0.001$ and $p < 0.05$, respectively). Of 4 further animals tested, 2 showed a partial recovery of REMS on hours 6 and 7 (49 % and 62 % of control), whereas in the others recovery was just beginning.

F Chemical determinations, Bioassay

1. Serotonin (5-HT)

After killing the rat by decapitation, the brain was removed, the hypothalamus dissected on an iced platform (Glowinski and Iversen, 1966) and stored for a few days at -20°C.
5-HT level of the hypothalamus was determined after butanol-heptane extraction according to the slightly modified fluorometric method of Curzon and Green (1970). Each sample was processed in duplicate.
Fluorescence was read on a Farrand Spectrofluorometer with excitation wavelength set at 360 nm and emission wave-length at 470 nm.

2. Delta Sleep Inducing Peptide (DSIP)

DSIP was quantitated by UV-spectroscopy at 280 nm and tested for purity on a Durrum D-500 amino acid analyzer.

3. Arginine vasotocin (AVT)

Thinlayer chromatography (TLC)

TLC was carried out on Silicagel plates F 254 (Merck, Darmstadt G.F.R.) Liquid phase: butanol : H_2O : HOAc, 3 : 1 : 1. The R_f-value for AVT was 0.12, one batch showing two spots, an impurity with an R_f-value of approx. 0.085. Since in this system the R_f-values were very low, the peptide was tested by a new and more accurate HPLC technique.

High-Pressure liquid chromatography (HPLC)

HPLC was carried out at 33°C on an Altex model 110A HPLC pump equipped with an Orlita pulse-dampner. 1 - 5 µl of a freshly prepared 0.1 % solution of AVT in 0.01 M acetic acid were injected via a Rheodyne mod. 7120 septumless valve with a 20 µl loop onto a 250 x 4 mm I.D. stainless steel column slurry-

packed with Nucleosil 10-C18 (Mackerey-Nagel, Düren, G.F.R.). (O'Hare and Nice, 1979).
Eluant (15 % acetonitrile in 0.1 M NaH_2PO_4-H_3PO_4 buffer, pH 2.8) was delivered at 1.0 ml/min (ca 3000 p.s.i.). Acetonitrile used was HPLC grade (Lichrosolv Merck, Darmstadt G.F.R.).
Buffer and salt solutions were made with pro analysi reagents (Merck) using double-glass distilled water. Eluted AVT was detected by UV-absorption at 225 nm on an UVIKON 725 variable wavelength spectrophotometer and at an attenuation range of 0.005 - 0.05 a.u.f.s. Retention time of solvent (acetic acid) was 2.55 min and of AVT 3.9 min. Detection limit was ca 50 ng AVT.
Impurities were found in one AVT batch with a retention time of 3.25, 5.3 and 5.6 min. The results obtained with this batch were discarded.

Determination of peptide loss through adherence

A 1 ml solution of 10^{-2} pg/µl 125-I -AVT in saline was put in four different types of vials: plastic, glass, plexiglass and soliconized glass. The entire solution was immediately removed with a pipette, and the empty vial as well as the removed solution including the pipette tip were counted for radioactivity on a γ-counter.
The loss through adherence to the vial was 27, 16, 26 and 15 % respectively. By rinsing the vials for 20 min with 1 ml saline the loss was reduced to 23, 11, 21 and 10 %, respectively.
In a second test ten siliconized vials were filled with 1 ml of the same solution and the experiment repeated. Mean count per vial was 1363 cpm $\pm$ 201.85 SD (30 % deviation). Before rinsing the mean loss was 13 $\pm$ 2.8 %, after rinsing 12.04 $\pm$ 3.67 %. Very little peptide was lost through adherence to the silicon vial.
To account for the larger surface of the glassware used in the rat experiments a further test was carried out. 10 µl were removed from a vial and the remaining solution was put into a new

vial. This procedure was repeated seven times. All vials were counted for radioactivity. The mean loss per vial amounted to 5 ± 4%.
A last test included the silicone tube (Silastic 0.02 in ID), infusion cannula and hamilton syringe used in the experiments. The AVT solution was left in the tubing for 10 min. Loss through adherence to the tube and the syringe was ca 20 %.

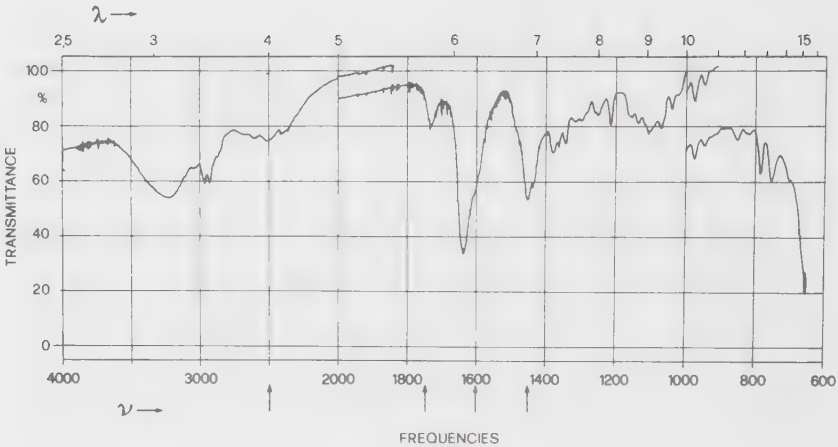
Bioassay

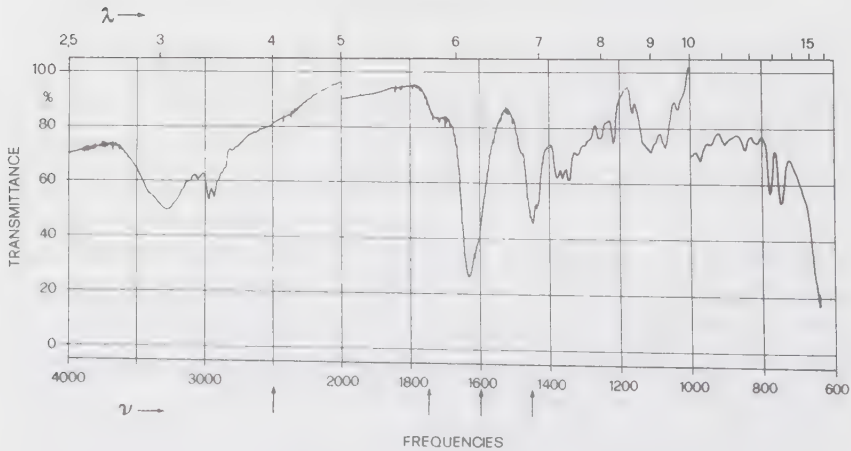
The effect of strongly diluted AVT solutions on water transfer across the wall of two isolated urinary bladders of the toad bufo marinus was tested according to the slightly modified method of Bentley (1958). The weight loss of the bladders expressed in % of control (control = 100 %) after a 30-min bath in Ringer solution with 2.5×10^{-1} pg/µl AVT was 132 % and with 1 pg/µl 234 % of control.

4. Lysergic acid diethylamide (LSD) and further substances

LSD used in the experiments was dissolved in double glass distilled water (LSD is very susceptible to traces of free halogenes). A stock solution of 400 µg LSD-tartrate in 1 ml water was prepared and stored at -20°C. A freshly prepared stock solution was subjected to infrared spectroscopy (IR) and compared to a two week and a two month old solution. Clear changes in the spectra could be observed in the older solution (Fig. 17). All experiments were carried out with freshly diluted stock solutions which had been stored for max. 3 weeks.
IR-spectra of physostigmine salicilate, atropine sulphate and methylatropinebromide solutions were compared with standard spectra of Sadtler Research Laboratories Inc.(1970). The spectra were identical. All experiments were carried out with freshly prepared solutions. Infrared attenuated total reflection spectroscopy (ATR) was performed with a Beckman IR-4. Some drops of the aequous test solution were spread on both sides of a zinc selenide ATR plate and the solvent evaporated (Harrick, 1967).

Figure 17:





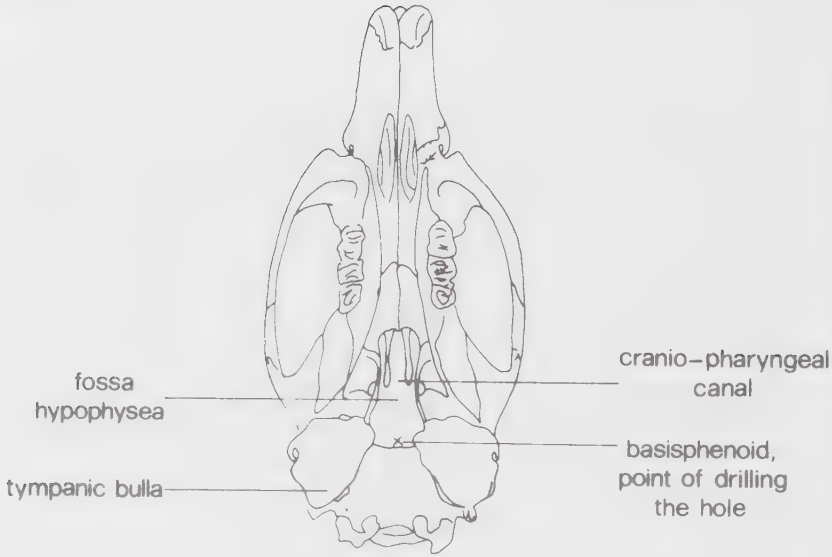
IR-spectrum of a freshly prepared LSD-tartrate solution (above) and of a two month old solution (below). Changes in the spectra at the following frequencies: 2500, 1730, 1610 and 1470 cm^{-1}.

G Further Methods

1. Hypophysectomy

Surgery was performed on male rats weighing 130-210 g. Under ether anesthesia the rat was immobilized on its back by means of wool string looped about its hind legs. The head and neck were slightly extended by a rubber band looped over the upper incisors. The operation was performed with the head of the animal towards the operator. During surgery the rats were warmed with an IR lamp. To avoid closing the trachea by the following manipulations, a blunted hypodermic needle (size 12) was inserted into the trachea. On some rats a tracheotomy was performed which proved to be an unnecessary intervention since a second person had to keep the opening clean.
A midline skin incision was made at the level of the thyroid. The salivary glands and subcutaneous tissue lying to the left were freed by blunt dissection and retracted to the right with a small hemostat. With the tips of a fine curved forceps the muscles were spread apart taking care not to damage the nerves and vessels of this region, until the forceps reached the midline exposing the crista occipitalis. This area was freed of muscle tissue and dried with cotton pellets before drilling. A hole was drilled with a No 9 (2.1 mm) dental burr in the midline of the occipito-sphenoid synchondrosis, taking care not to rupture the membranes covering the pituitary. (Fig. 18)
The edges of the drill hole were cleaned and the dura torn away with a fine hook, thus exposing the pituitary. Usually this procedure was followed by bleeding. A pipette with an opening just fitting into the drill hole was placed against the pituitary. The gland was removed by suction that was just strong enough to draw the gland into the pipette. Suction was accomplished by a vacuum pump and a suction trap which allowed to collect and visualize the removed tissue. When properly removed, the anterior lobe was broken into two parts, and the intact posterior lobe was separated from the anterior lobe. A second

Figure 18:



Basal aspect of the rat skull

suction usually caused strong bleeding and resulted in death of the animal.

The exposed tissues were wiped clean and the skin incision glued with collodium. The short duration of the operation proved to be important for the post-operative survival, an ideal time being 5 - 10 min per animal (which takes a great deal of practice).

For post-operative care the rats were kept at a temperature of approx. 28°C. They were handled as little as possible to avoid unnecessary stress. No salt or sucrose was added to the drinking water as is often recommended.

A recovery of at least ten days was allowed before electrodes were implanted for sleep recordings. This second surgery was carried out under ether anesthesia, and led to the death of

30 % of the hypophysectomized rats.
Very often respiratory movements ceased and had to be restored by air blown into a rubber tube held over the nose of the animal, as well as by applying rhythmically, pressure on the thorax. The criteria for a successful hypophysectomy were the arrest of normal growth and an empty sella turcica which was controlled after sacrificing the rats.

2. Aspiration of cerebrospinal fluid (CSF)

The rats were heavily anesthetized with ether. The cisterna magna was layed free and punctured by a very fine hamilton syringe needle with a slanted sharp tip. Usually it was possible to aspirate approx. 60 - 100 µl fluid until blood appeared. The last fraction containing blood was discarded. Pressure on the abdomen increased the volume that could be aspirated.
CSF from three rats was pooled in a small vial and exposed to reduced pressure for 15 min to evacuate the ether. CSF was either used immediately or stored at -20°C.

3. Gas chromatography (GC) determination of ether in CSF

A quantitative GC determination of ether in blood plasma, in freshly aspirated CSF and in CSF exposed to 5 or 15 min low pressure was carried out to estimate the contamination due to ether anesthesia.
GC conditions: glass column (160 x 0.3 cm i.d.) filled with Porpak Q (100/120 mesh) (Waters Assoc. Inc. Milford.,Mass.USA); flame ionisation detector; column temperature 108°C; injector temperature 195°C; carrier gas nitrogen, 25 ml/min. The CSF extracts were diluted with n-hexane and injected into the GC column. Injection volume 1 µl. The range of the calibration curve for ether was 20 ng - 1 µg/mg per sample. Untreated CSF contained 0.05 - 0.07 % ether, vacuum treated samples contained 0.004 - 0.009 % ether. No difference in ether concentration was

found between 5 or 10 min vacuum aspiration. Blood plasma contained 0.03 % ether.

III REM-SLEEP-INDUCTION BY SHORT DARK OR SHORT LIGHT PERIODS

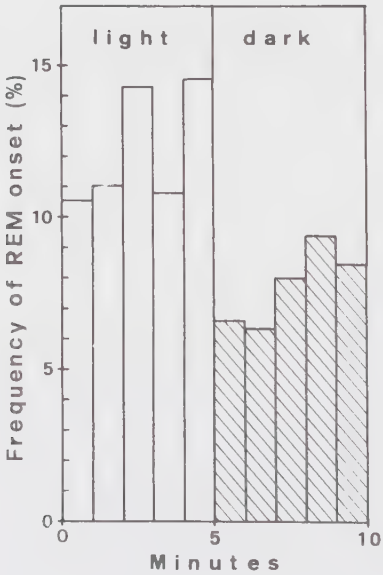
The circadian activity-rest or activity-sleep rhythm of rats is strongly dependent of environmental lighting conditions. When rats are kept under a 12 h light (L) - 12 h dark (D) schedule they are mainly active during the dark period and mostly asleep or inactive during the light period. This is a very convenient rhythm for the sleep researcher who may thus observe the sleeping animal in the laboratory during daytime hours. In most of our studies the animals were entrained to a 12-12 h LD schedule since the distribution of vigilance states during this schedule has been well documented. (Borbély, 1978a).
However, Webb and Friedman (1971) showed that sleep patterns of rats are resistent to environmental variations. Nevertheless short LD-cycles have a selective effect on the sleep states of the rat. REMS episodes are redistributed so that most of them occur during the dark periods, while the total amount of REMS and NRMS remains unaffected. This unique action of an environmental condition on a sleep state was discovered by Lisk and Sawyer (1966) who reported that in the female albino rat maintained under a 25 min light-5 min dark schedule (LD 25:5 min), most REMS-episodes occurred during the dark periods. This finding was confirmed for LD-cycles ranging from 2.5:2.5 min to 6:6 h (Rechtschaffen et al., 1969; Johnson et al., 1970; Borbély et al., 1975; Satinoff et al., 1975; Borbély, 1976). Attempts to document such a selective action of lighting conditions on REMS in species other than the albino rat (cat and ground squirrel) have not been successful (Chamblin and Drew, 1971; Satinoff et al., 1975 and Lucas, 1979). Recently Oliverio

and Malorni (1979) reported an enhancement of total sleep during the light period in two mouse strains subjected to short LD-cycles (6:6, 3:3, 1:1 h). No effect on REMS was found.
In our study we tested (i) a further rodent species, the golden hamster, who is widely used in rhythm research, and (ii) a non-albino rat strain. Numerous studies have documented the precision and stability of hamster circadian locomotor activity rhythm (Aschoff et al., 1973) which is free-running in the absence of an entraining agent. We exposed the hamsters to short LD-cycles to see if their distribution of sleep states could be manipulated. The LD 5:5 min or LD 10:10 min were chosen because these LD-cycles had proven highly effective in demonstrating a dark-induction of REMS in the rat (Borbély, 1976).
The mechanisms underlying the selective effect of short light or dark periods is still obscure. With the following experiments we investigated the role of two factors that seemed to be involved on the basis of previous studies. The amount of REMS-episodes in the rat during short dark periods was reported to depend on the intensity of the preceding light period (Rechtschaffen et al., 1969). Since in all previous experiments highly light sensitive albino rats have been used, we studied the effect of short LD-cycles on the sleep state of a non-albino strain. A second series of experiments in which we investigated the role of the pituitary gland, was prompted by a recent report by Stern and coworkers (1976) that hypophysectomy abolished the redistribution of REMS to dark periods in the rat. These results seemed to implicate pituitary hormones in the mediation of sleep changes by short LD-cycles, whereas the ablation of the other endocrine glands such as the pineal gland, the ovaries (Johnson et al., 1970) and the adrenal glands (Johnson and Sawyer, 1971) has proved ineffective.

A Effect in the golden hamster

Experimental subjects were adult male golden hamsters (Mesocricetus auratus; body weight 139 - 194 g) kept under LD 12:12 h or LD 14:10 h conditions. Electrodes were implanted as described for rats in p. 16.
The hamsters were exposed to LD 5:5 min or LD 10:10 min cycles during 2 to 3 daily recording sessions lasting 1 to 3 hours during their daylight hours.
In the golden hamster REMS predominated during the light periods, whereas NREMS showed practically an equal distribution between light and dark periods. (Table 4, Fig. 19).

Figure 19:



Onset frequency of REMS during 1-minute periods of a 5 min light - 5 min dark cycle in the golden hamster. Frequencies are expressed as the percentage of all REMS-onsets (N = 426; 8 animals, 5 - 9 recording sessions per animal). A separate REMS-onset was counted if it occurred at least 20 sec after the preceding REMS-episode.

Table 4: Sleep states of the golden hamster during short light (L) and dark (D) periods.

Mean values (SEM in parenthesis). Data from 8 animals. REMS:REM sleep, NREMS: nonREM sleep. LD 5:5 min : 5-9 recording sessions per animal. Total frequency of REMS-onset: 426; LD 10:10 min : 2 recording sessions per animal. Total frequency of REMS-onset: 121.

Light-dark cycle (min)	Frequency of REMS-onset (% of total)		Sleep state (% of recording time)			
			REMS		NREMS	
	L	D	L	D	L	D
5: 5	61.3[a]	38.7	9.9 (0.85)	8.5 (0.26)	31.5 (1.36)	31.8 (0.79)
10:10	57.9[b]	42.1	11.2[c] (1.59)	7.5 (1.11)	31.4 (2.85)	30.9 (1.36)

a $p < 0.01$ compared with D; one-sided sign test with pooled values, or one-sided paired Wilcoxon-test with values of individual animals.

b $p < 0.05$ compared with D; one-sided sign test; $p = 0.05$ with one-sided paired Wilcoxon-test with values of individual animals.

c $p < 0.05$ compared with D; one-sided paired Wilcoxon-test with values of individual animals.

Other differences were non-significant ($p > 0.1$).

It should be noted that the onset frequency of REMS during the light periods was higher with the LD 5:5 min schedule, whereas the percentage of REMS was higher with the LD 10:10 min schedule. This may indicate that light has both a REMS-inducing effect (more evident with frequent, short light periods), and a REMS-maintaining action (more evident with longer light periods).
Thus the enhancement of REMS by short periods of light or darkness is not limited to the albino rat, but extends to at least one more species. It is puzzling though that in the golden hamster REMS predominates during light periods, whereas in the rat REMS is enhanced by dark periods. Both species are nocturnal and exhibit a similar distribution of their vigilance states (Van Twyver, 1969). In a nocturnal animal one would expect the absence of light to be a very important alerting stimulus. A major difference between the two species is the fact that the hamster is a hibernator. It is unclear, however, how this difference could be relevant for the enhancement of REMS by different lighting conditions.

B Effect in two rat strains

Five male rats of the albino Sprague-Dawley/Ivanovas strain (SIV-50) were compared with six rats of the non-albino strain DA. Experimental conditions are described in p. 18.
Surgery, recordings and computations were carried out as described in methods: p. 15. Three of the 5 albino rats weighed only 120-160 g to serve as controls for the hypophysectomized rats. The rats were kept under LD 12:12 h conditions (light from 7:00 to 19:00). They were exposed to LD 5:5 min, 10:10 min or 15:15 min cycles during two recording sessions lasting 2-4 h each, during the light-phase of the LD 12:12 h cycle.
In the albino rats exposed to short LD-cycles REMS was redistributed so that about two thirds of the total frequency and time of REMS-episodes occurred during the dark periods (Table 5).

Table 5: Distribution of sleep during short light-dark (L, D) cycles in intact and hypophysectomized albino rats and in the non-albino DA rat strain.

Light-Dark cycle (min.)	Animals	Frequency of REMS-onset (% of total)		Sleep state (% of recording time) REMS		Sleep state (% of recording time) NREMS		N
		L	D	L	D	L	D	
5:5	Intact	32.3	67.7*	7.2	16.3*	37.7	32.4	5
10:10		28.5	71.5**	4.2	12.2***	33.4	25.0*	5
15:15		36.3	63.7**	6.3	11.5**	32.7	30.5	5
5:5	Intact	43.6	56.4*	7.5	8.5	34.4	34.1	6
10:10	DA	37.5	62.5*	6.1	9.2**	32.9	30.0	4
5:5	Hypophysectomized	28.3	71.7**	3.5	11.2***	37.2	33.0	10
10:10		30.2	69.8**	3.4	10.0***	34.9	29.8**	10
30:30		36.9	63.1	3.9	7.0*	34.7	33.1	2

One-sided paired t-test was performed to evaluate the difference between light and dark periods (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). N = number of animals, REMS: REM sleep, NREMS: nonREM sleep.

Since no difference was seen between the young and adult intact SIV-50 rats, the data from the two groups have been pooled. A redistribution of REMS to the dark periods was present also in the non-albino rats, although this effect was weaker than in the albino rats. The difference between the two strains was statistically significant for LD 5:5 min (frequency of REMS-episodes) and LD 10:10 min (percent of recording time) ($p < 0.05$; two-sided Mann Whitney test).
The present experiments showed that the albino condition is not a prerequisite for the redistribution of REMS to the dark periods. The similarity of the sleep patterns of albino and pigmented rat strains has been pointed out previously for the LD 12:12 h condition (Van Twyver et al., 1973). Since in the albino rat the predominance of REMS-episodes during short dark periods has been shown to be a function of the intensity of the preceding light period (Rechtschaffen et al., 1969), strain differences in light sensitivity could account for the observation that REMS was more concentrated to the dark periods in the albino rats than in the pigmented strains.

C Effect in hypophysectomized rats

Experiments with hypophysectomized rats were conducted with 10 Sprague-Dawley rats weighing 130-210 g at the time of hypophysectomy. Since Valatx (Valatx et al., 1975) reported, that a stable baseline of sleep was not obtained earlier than 3 weeks after ablation of the pituitary gland, we allowed 3 weeks for recovery, further surgery and adaptation to the recording chamber before we started recording. The rats were exposed to similar short LD cycles as described before, LD 5:5 min, 10:10 min and 30:30 min.
Hypophysectomized albino rats showed a clear predominance of REMS-episodes and total REMS-time in the dark periods, and did not differ significantly from the intact controls (Table 5). We were therefore unable to confirm the findings by Stern and

coworkers (1976) that the redistribution of REMS is abolished by hypophysectomy. We are unable to advance plausible arguments for the failure to replicate their results. A damage of the optic pathways and the suprachiasmatic region which could occur during hypophysectomy, has been apparently ruled out by Stern and associates. It is also unlikely that the length of the LD-cycle is a decisive factor.
We performed 2 experiments in hypophysectomized animals with the LD 30:30 min schedule used in the previous study, and obtained essentially the same results as with shorter cycles (Table 5). We conclude therefore from our experiments that the presence of the pituitary gland is not a prerequisite for the redistribution of REMS to short dark cycles in the rat. Even though the mechanisms of action remain unknown, our findings provide further evidence that light, a natural environmental variable, may exert rapid and selective effects on vigilance states, and thus constitutes a powerful experimental tool in sleep research.

IV NEUROTRANSMITTERS AND SLEEP REGULATION

M. Jouvet, the "father" of the monoaminergic sleep theory states that the 5-HT containing perikarya of the raphe system play a determinant role in the induction of sleep (Jouvet,1977). A thorough study, however, of the relationships between monoamines (MA), acetylcholine (ACh) and the sleep-waking cycle has only recently become possible, since chronic polygraphic recordings of the sleep state, combined with the anatomical delineation of MA systems, and modern biochemical techniques were necessary for a multidisciplinary approach to the problem.
As Jouvet states in his reviews (1972, 1977), destruction of the raphe nuclei, the decrease of 5-HT in the nerve terminals

and insomnia are closely correlated. The norepinephrine (NE) containing neurons of the locus coeruleus are involved in the maintenance of tonic cortical arousal (in addition to cholinergic ascending mechanisms). The dopaminergic nigrostriatal system is involved in the maintenance of waking behavior. According to Jouvet's monoaminergic theory there are reciprocal interactions between the 5-HT and NE-systems. This theory emphasizes the importance of the brainstem where the MA perikarya are located, although various brain levels at which an interaction between the 5-HT, catecholamine and ACh terminals occurs, are responsible for the behavioral and EEG aspects of the sleep-waking cycle.
It is possible that some postsynaptic system activated by MA or by ACh might synthesize some polypeptides or even proteins which would be the final messengers of sleep. However, a purely humoral theory of sleep does not seem to explain satisfactorily the central mechanisms involved in the regulation of sleep and waking. (Jouvet, 1977).

A Serotonergic system and sleep

Research with the monoamine 5-HT is complicated by the fact that 5-HT is unable to cross the blood-brain barrier (Costa and Aprison, 1958) in mammals. Palaič and coworkers (1967) showed that 5-HT is removed from CSF by brain uptake, adding a new methodical aspect to the study of the role of 5-HT in sleep. Cerebroventricular infusion of 5-HT in the cat induced sedation and cortical synchronization (Fuxe and Owran, 1965). Furthermore, infusions of 5-HT in rats produced depressing effects on locomotor activity resembling those of CSF from sleep deprived goats (Fencl et al., 1971; Green, 1976). The depressant effects of 5-HTP, the direct precursor of 5-HT, on behavior are well known (Monnier and Tissot, 1958; Delorme, 1966). With our experiments we intended to test the effect of cerebroventricular 5-HT administration on sleep in the rat.

Reports on LSD, a substance with central serotonin agonist and antagonist properties (Bennett + Snyder, 1975) are contradictory and seem to differ largely depending on the species tested, on the route of application and on the dosis applied. LSD decreases 5-HT turnover and blocks firing in the dorsal raphe nucleus (Aghajanian and Weiss, 1968; Aghajanian et al., 1968, 1970; Haigler and Aghajanian, 1974). Hobson (1964) found an increased cortical synchronization and suppression of REMS in the cat. Relatively low LSD concentrations had no significant effect on sleep distribution in the rat (Bilkova et al., 1971), while on the other hand, Hartmann (1967, 1969) reported an increase of REMS after i.p. application in this species. Also an increase of REMS in human subjects was reported by Muzio et al. (1966) and Green (1969).
We tested sleep in rats after a short i.v. infusion of small LSD doses.

1. Serotonin: Effect of intraventricular infusion on sleep

Ten rats were infused in the lateral ventricle with aCSF-II (p. 36), containing 5, 0.5 or 0.05 µg/min 5-HT. All infusions were given at midday and lasted approx. one hour unless the rat woke up and was active for more than ten consecutive minutes. In this case only the periods prior to waking were used for the computations. Infusion rate was ca 3 µl/min. If the animal did not lose weight from one day to the other, it received an infusion on several consecutive days, each day beginning with a control session without infusion. With the two higher doses (5 µg/min and 0.5 µg/min) the rats woke up abruptly after a few minutes of infusion, and remained active for a prolonged period. Of 12 infusions with 0.05 µg/min, 7 had to be interrupted due to arousal. There was no significant difference in any of the scored vigilance states compared to aCSF infusions.

2. Lysergic acid diethylamide (LSD): Effect of intravenous infusions on sleep

Thirteen rats were implanted with EEG electrodes and a chronic cannula in the jugular vein (p. 26). The sleeping rats were infused with saline for 1 - 2 hours before they were infused with the drug for approx. 1 min (p. 29). Each animal was infused with 2 - 4 drug concentrations on consecutive days: 0.3, 3 - 5, 20, 30 and 40 µg/kg LSD-tartrate (concentrations are given for the free base). Sleep was recorded for 30 min before the drug infusion and for 30 - 60 min after the infusion. Sleep stages were scored according to the method described on p. 21. In six rats various 10-s periods of the EEG were subjected to frequency analysis before and after infusion of LSD 0.3, 3 and 10 µg/kg. No difference was found in the distribution of any of the vigilance states scored or in the EEG frequencies.

3. Discussion

In contrast to the arousing effect of 5-HT in our experiments, Green and coworkers reported no enhancement of motor activity following injection of 1-50 µg 5-HT into the lateral ventricle (Green et al., 1976). This suggests that the arousal effect is not necessarily followed by enhanced motor activity, and may be evident only when infusing 5-HT into the ventricle of the sleeping animal. Our findings correspond with Koella's reports on cats which were aroused after i.v. injection of 5-HT. (Koella and Czicman, 1966).

It is difficult to forward an explanation for the arousal effect. It must be taken into consideration that the infused 5-HT may not be taken up by the specific terminals in the ventricular lumen. Infused 5-HT may mimic the effect of endogenous 5-HT at the receptor site if it reaches these receptors and if the amount is sufficiently small. However, it may also exert a pharmacological effect upon other cells since it may be taken

up by other MA neurons (Aghajanian and Bloom, 1966; Fuxe and Ungerstedt, 1968).
The dose seems to be an exceedingly important parameter for LSD experiments. Aghajanian and Haigler (1975) have shown that low doses of LSD (10 - 30 µg/kg i.v.) in the rat have an almost exclusively presynaptic action, while higher doses begin to have a postsynaptic serotonin agonist effect. Trulson and coworkers (1976) have shown that doses of LSD greater than 400 µg/kg i.p. have a potent 5-HT agonist action in the rat. Bilkova and coworkers (1971) found an increase in frequency of hippocampal theta activity during REMS, which would agree with an activating effect of this drug upon the ascending reticular activating system. However, if low LSD doses disinhibit target neurons, the decreased raphe firing alone may not be sufficient to trigger sleep. Many pharmacological substances are reported to decrease raphe firing which have not been reported to enhance sleep (e.g. Aghajanian et al., 1968 and 1970).

4. Parachlorophenylalanine (PCPA)

PCPA is a most potent tryptophanhydroxylase inhibitor. It decreases 5-HT in the rat brain while causing only a minor reduction in the level of catecholamines (Koe and Weissmann,1966; Gal et al., 1970). Thus PCPA is an ideal drug to manipulate sleep mechanisms by interfering specifically with the synthesis of 5-HT, and offering thereby a unique approach to the problem of the role of 5-HT in sleep regulation.
Reports on the effect of PCPA administration in the cat and in the rat show a depression of sleep (Delorme et al., 1966; Koella et al., 1968; Mouret et al., 1968; Rechtschaffen et al., 1973), which can be reversed for a short time with the serotonin precursor 5-hydroxytryptophan (5-HTP), (Mouret et al., 1967; Koella et al., 1968).
Since PCPA inhibits tryptophanhydroxylase the rate limiting enzyme in 5-HT synthesis, we tested the possibility of reversing

PCPA induced changes of sleep, behavior and brain 5-HT concentration by administering tryptophan (TRP).
The timecourse of the effect of PCPA on the brain 5-HT level has been documented in a previous study from our laboratory (Steigrad et al., 1978). In the present experiments we measured forebrain 5-HT levels after TRP application in PCPA pretreated animals.
The studies investigating the effect of PCPA on sleep generally used a systemic administration of the drug. A study with cerebroventricular application of PCPA centered on the effect of feeding (Breisch et al., 1976). The authors reported that 3 days after drug infusion the rats displayed a marked hyperphagia which lasted for 1-2 weeks.
The following experiments were undertaken to clarify the effect of central PCPA administration on sleep, following a preliminary study of motor activity in which the weight of the animals was carefully monitored.

Effect of cerebroventricular PCPA-administration on motor activity

Nine rats with a chronically implanted cannula in the lateral ventricle (p. 31) were infused either with saline (12 µl, controls), or with 2 mg (8 µl), 3 mg (12 µl) or 5 mg (12 µl) PCPA (PCPA-methylester, all doses are given for the acid) after 1 - 2 days control registration. (Infusion rate approx. 1 µl/min). On day 2 they were infused 30 min before dark onset (D: 19 - 7 h), and registration was continued for 3 - 6 days. The 3 rats infused with 5 mg PCPA died suddenly during or immediately after infusion.
In all animals motor activity was reduced for 6 - 8 h after the infusion. However, on the following days, 2 PCPA rats showed a marked dose dependent hyperactivity during the dark-period, with practically no change during the light-period, whereas the other 2 PCPA rats showed a marked general change in the motor activity pattern. Their dark-period was characterized by long

lasting activity bouts followed by periods of rest. In these animals daytime activity was enhanced (Fig. 20). This effect persisted during the 6 days of registration following the PCPA infusion.

Effect of PCPA on body weight

Under light ether anesthesia 12 rats were infused through an acute cannula in the lateral ventricle with 8 - 12 µl saline (n = 2), PCPA 2 mg (n = 2) or 3 mg (n = 8), 30 min before dark onset. Body weight was measured for 5 - 7 days after the infusion.
No tendency to hyperphagia or enhancement of body weight was found.

5. Effect of tryptophan (TRP) on PCPA-pretreated rats

Sleep

Three rats were recorded by telemetry during the light-phase (10 - 22 h) of a control day and of the day starting 24 h after administration of PCPA (300 mg/kg i.p.). On this second registration day they were injected at 14:00 h with l-tryptophan (Fluka, Buchs, Switzerland; TRP suspended in saline with 0.5 % Tween-80). The EEG was recorded on magnetic tape and subsequently subjected to spectral analysis (p. 22).
Fig. 22 illustrates the effect of the tryptophan injection in one rat as compared to a control day. There is a marked reduction in motor activity and an increase in sleep. The delta activity of NREMS reappeared after TRP injection and increased in size for 1 - 2 hours. REMS which was markedly depressed one day after PCPA, is clearly enhanced for a few hours after the injection, the reappearance of a regular NREMS/REMS cycle being reflected by the equal intervals between the delta peaks, and by the theta peaks (6.25 - 4.50 Hz band). A slight but less dramatic rise of the highest frequency band (10.25 - 15.00 Hz) whose power is mainly constituted by spindle activity, can also be seen.

Figure 20:

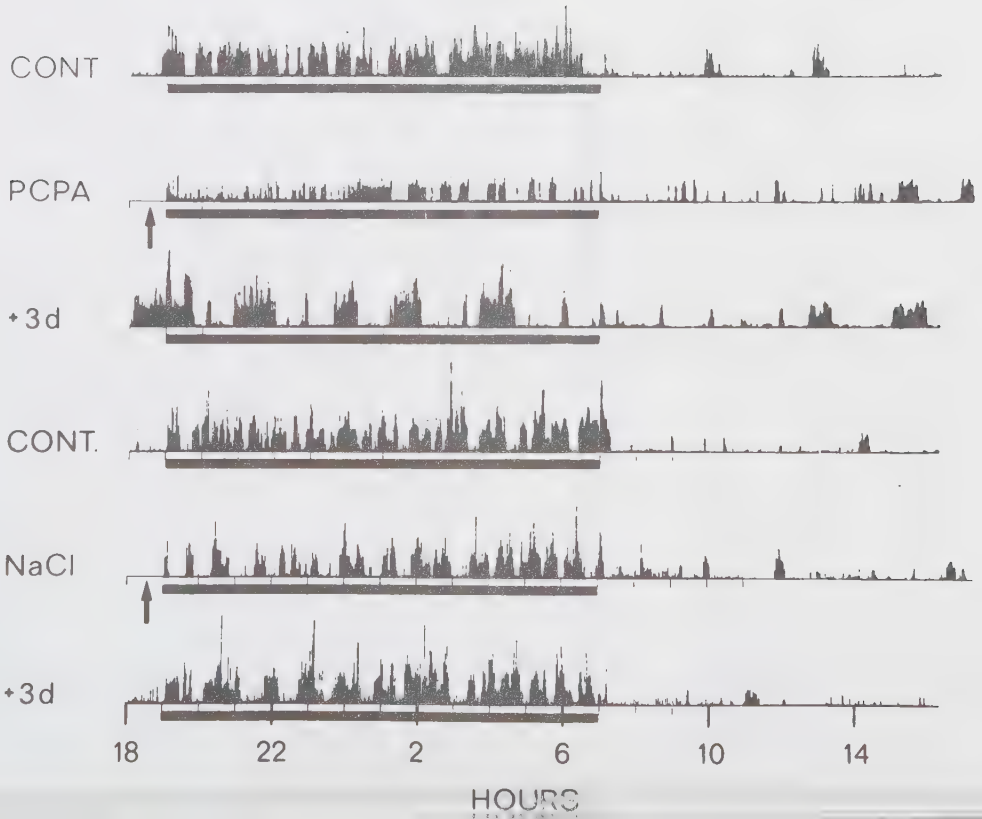


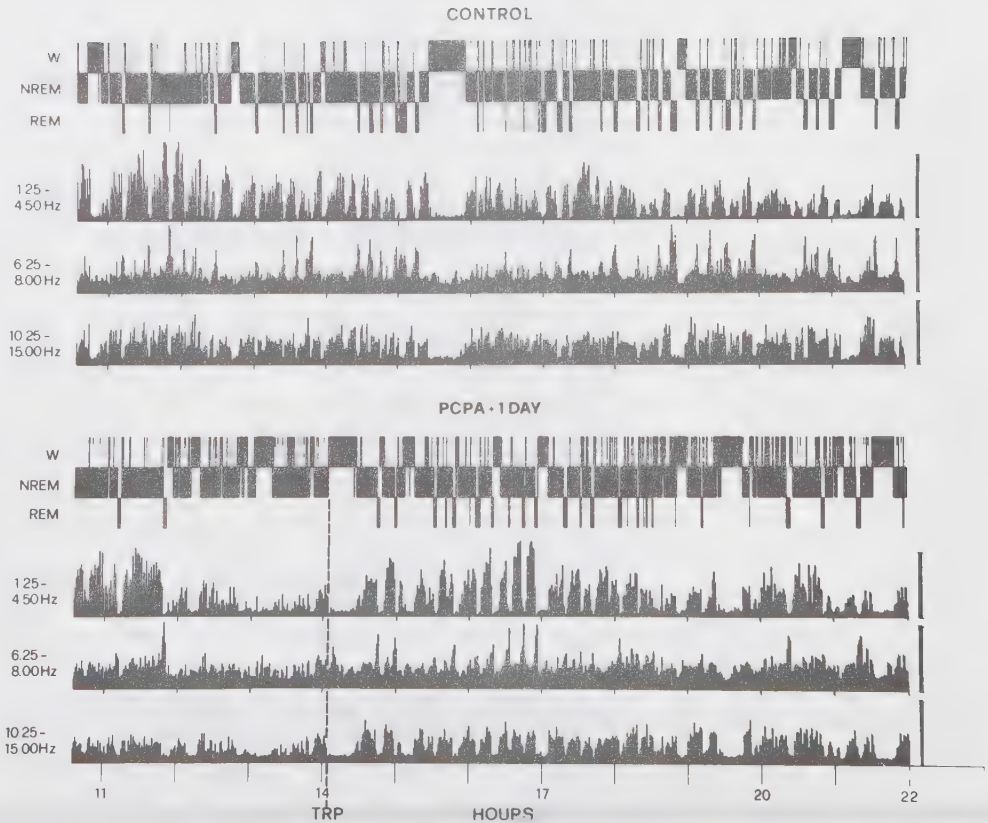
Figure 20:

Motor activity of 2 rats, on a control day, one day immediately following infusion of PCPA (3 mg/12 µl) and NaCl (12 µl), and on the third day after the infusion. Dark-phase from 19 - 7 h indicated by bars beneath the records. Arrows indicate time of infusion. Motor activity is plotted in arbitrary units.

Figure 22:

Vigilance states and EEG power-spectra in a rat recorded during the light-phase of the control day (top) and one day after PCPA-administration (bottom). Vertical line at approximately 14 h indicates injection of l-tryptophan (TRP; 150 mg/kg i.p.). The calibration marks on the right represent 200 $\mu V^2/0.25$ Hz for the low and medium frequency bands and 80 $\mu V^2/0.25$ Hz for the high frequency band.
Vigilance states are plotted for successive 20-s epochs, the spectra of the 3 frequency bands indicated on the left are plotted for 1-min epochs. The spectra are means of 15 successive values computed for 4-s epochs, and means of several adjacent 0.25 Hz frequency bands. Hours on abscissa indicate time of day.

Figure 22:


CONTROL
W
NREM
REM
1.25 - 4.50 Hz
6.25 - 8.00 Hz
10.25 - 15.00 Hz
PCPA - 1 DAY
W
NREM
REM
1.25 - 4.50 Hz
6.25 - 8.00 Hz
10.25 - 15.00 Hz
11
14
17
20
22
TRP
HOURS

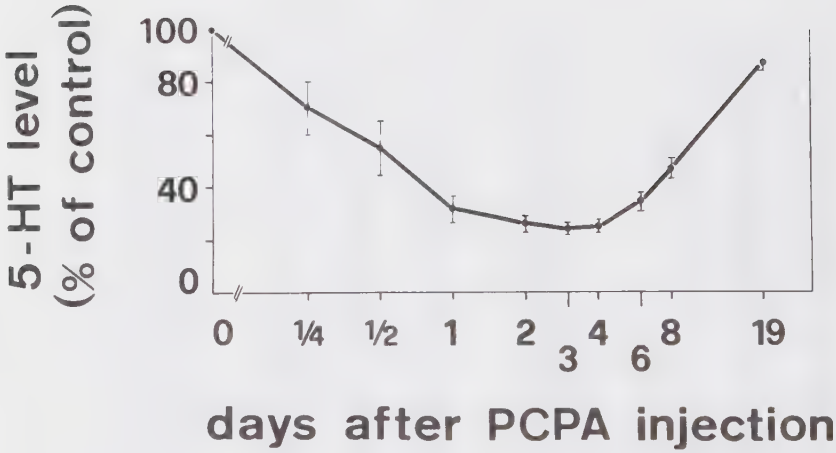
Forebrain serotonin concentration

In 12 rats the effect of TRP on forebrain 5-HT concentration after PCPA pretreatment was investigated. All animals were administered PCPA (300 mg/kg i.p.). Twenty-four hours later one group was injected with TRP (150 mg/kg i.p., n = 6), the other with saline-Tween solution (controls, n = 6). One hour after drug administration the rats were decapitated, their brains removed, and forebrain 5-HT determined according to the method described on p. 51.
The TRP group had significantly higher forebrain 5-HT levels than the control group ($p < 0.01$; 2-sided t-test). The values with SEM for nmol/g 5-HT in controls was 1.31 $\pm$ 0.40 and for the TRP group 2.08 $\pm$ 0.29.
Compared to the 5-HT levels one day after PCPA (300 mg/kg i.p.) (Fig. 21) and expressed in percent of levels of untreated rats, the 5-HT level after TRP was 61 %, and without TRP 38 %.

Discussion

Since in our hands PCPA administered into the lateral ventricle did not influence body weight, we were unable to confirm the results of Breisch et al. (1976). PCPA seems to have a toxic pharmacological effect, since the doses used were close to the lethal dose. Administration of either NaCl or PCPA had a long-lasting sedative effect in the first hours after infusion, which is comparable to the results from experiment p. 50, Fig. 14. Some effects of cerebroventricular PCPA administration are similar to those seen after systemic administration. Thus the temporal activity pattern was modified and the circadian rhythm attenuated (see Borbély et al., 1973). Although the light-time activity after both saline and PCPA infusion was higher than on the control day, activity was more increased by PCPA than by saline (Fig. 20). After systemic administration, motor activity during the first 24 hours is reduced to 80 % of control (Steigrad et al., 1978). However, one has to consider that PCPA was injected during day-time hours and not immediately before dark-

Figure 21:



Serotonin concentration in rat forebrain determined at various timepoints after injection of p-chlorophenylalanine (PCPA; 300 mg/kg i.p.). The points represent arithmetic mean values (± SEM; 4 - 7 rats) and are expressed as a percentage of the daily control values that were determined concomitantly with the PCPA values. The absolute control values for the 5-HT level is 3.0 ± 0.16 nmol/g (n = 7). Note the logarithmic time scale.

onset. Twenty-four hours after systemic PCPA administration in the beginning of the light-phase, motor activity was also enhanced (Borbély et al., 1980).

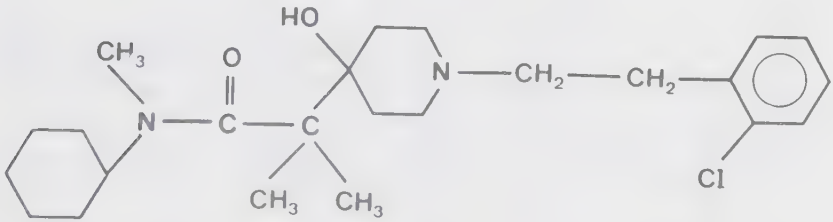
Both intraventricular PCPA doses had a very long-lasting effect on motor activity. After 6 days there was still no recovery, even though the effect of 300 mg/kg administered intraperitoneally lasted only 2 - 3 days. The long pharmacological effect after intraventricular administration might correlate with the timecourse of 5-HT depletion or represent a nonspecific action.

TRP, by raising the cerebral 5-HT level, restored the PCPA-disrupted sleep levels, although it did not alter normal sleep when given to control rats (unpublished observations) even though it raised their brain 5-HT levels. (Knott and Curzon, 1974). Thus PCPA seems to disrupt a neurochemical process responsible for physiological sleep regulation which is temporarily restored by tryptophan.

6. Effect of a serotonin antagonist (FU 29-245) on sleep in the rat

This substance is the methane sulfonate salt of 2 - (1-0-Chlorophenethyl-4-OH-4-piperidyl)-N-cyclohexyl-N-methyl-isobutyramide with the following structural formula:

Figure 23:



It has been shown to have antidepressant and analgesic effects after oral and parenteral administration (Sandoz Ltd., 1977). There are indications that FU possesses central serotonin antagonistic properties, and accelerates 5-HT metabolism in the rat brain in vivo. In behavioral tests with mice it caused a dose dependent inhibition of motor activity and an overall sedation. Sleep tests with human subjects given FU p.o. before going to sleep showed a specific enhancement of slow wave sleep (SWS), specially stage 4 sleep, with no reduction of REMS (Spiegel, 1979).

There are often broad species differences in the effects of

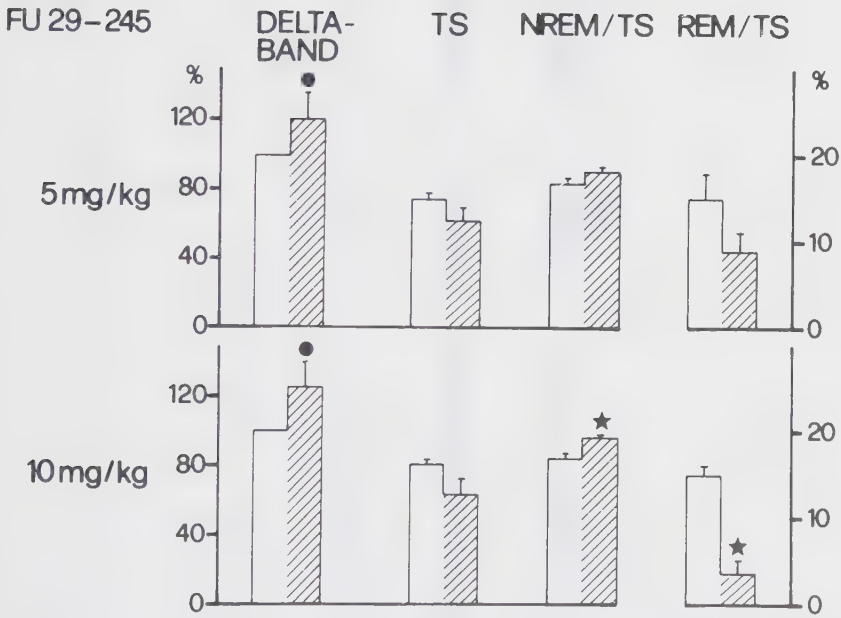
5-HT-antagonists on sleep. REMS, NREMS or SWS are reduced depending on the species tested. In our studies we wanted to see if the effect of FU on sleep in humans could be reproduced in the rat.

Sleep was recorded in seven rats (p. 15), and the sleep stages and the delta band were plotted (p. 22). Fig. 24. Each animal was recorded for a 1 - 2 h control period, then injected i.p. with 5 mg/kg (n = 3) or 10 mg/kg (n = 4) FU 29-245 (dissolved in distilled water), and finally recorded for another 2 - 3 h. Figure 25 illustrates the spectral power of the EEG of a rat. (p. 80). After injection the rats were awake for approx. 20 - 30 min. This prolonged waking was followed by a long and uninterrupted sleep in which delta waves predominated. Fig. 25. The alternation of waking and sleep recurred once or twice, and the effect disappeared after 2 h. A significant difference was found in the power of the delta band before and after FU 29-245 for the two drug doses combined ($n = 7$; $p < 0.05$; two-sided paired t-test), although the differences did not reach the level of significance for the individual doses. There was no significant difference in total sleep, NREMS and REMS between control and FU 29-245 5 mg/kg; however, a significant reduction of REMS was found after 10 mg/kg. ($p < 0.005$; two-sided paired t-test). Fig. 24.

B Cholinergic system and sleep

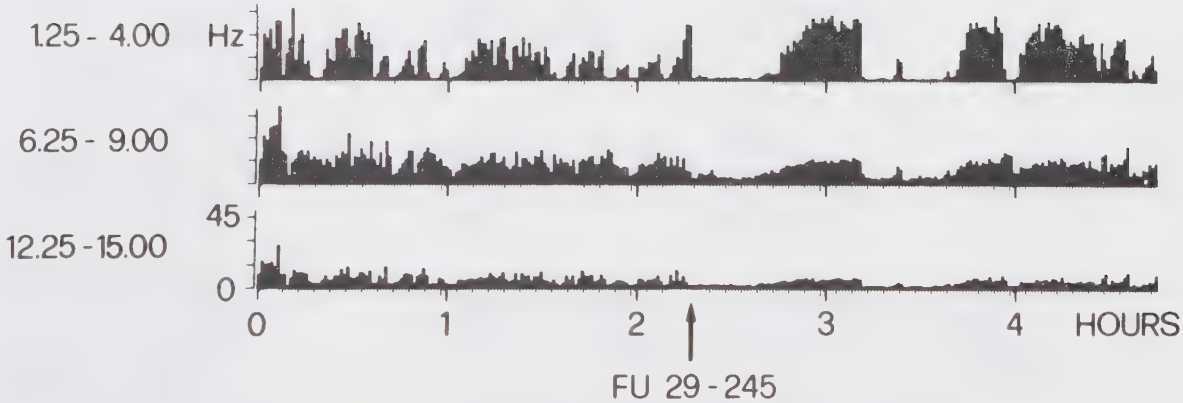
Research on biochemical control mechanisms of the three vigilance states has focused mainly on the role of biogenic amines (5-HT, NE). Nevertheless, there is increasing evidence, that REMS may be controlled by cholinergic interactions. Hobson et al. (1975) have proposed a neurophysiological model for the control of desynchronized sleep based on their studies of FTG cells (pontine gigantocellular tegmental field) and cells in the locus coeruleus and subcoeruleus in the cat. They suggest that a reciprocal interaction between these cells mediates REMS.

Figure 24:



Effect of i.p. injection of FU 29-245 on sleep. White bars represent pre-injection control values, hatched bars values after FU 29-245 (dose indicated on the left). Delta-band: spectral power per NREMS, expressed as percentage of control (control = 100 %, n = 3-4). TS: total sleep is expressed as percentage of recording time; NREMS and REMS are expressed relative to TS. Right ordinate applies only to REM/TS. Mean values with SEM. Asterisks: significant differences from control value ($p < 0.005$; two-sided paired t-test), dots: significant difference of the combined FU doses from control value ($n = 7$; $p < 0.05$; two-sided paired t-test).

Figure 25:



Spectral power density $((\mu V_{RMS})^2 / 0.25\ Hz)$ of the EEG in the delta band (1.25 - 4.0 Hz), and in the 6.25 - 9.00 Hz and 12.25 - 15.00 Hz bands before and after i.p. injection of 10 mg/kg FU 29-245. The power spectrum is plotted for 1 min periods, the arrow shows the time of injection.

REMS is induced by application of carbachol (a long-acting cholinomimetic drug) and physostigmine to the brainstem area in normal cats. (Baxter, 1969; Hernández-Peón and O'Flaherty, 1967).

Physostigmine a short-lasting anticholinesterase agent which crosses the blood brain barrier, increases central as well as peripheral cholinergic actions. In order to block its peripheral cholinergic effects it is often combined with methyl-atropine or methyl-scopolamine, peripheral anticholinergic agents that barely cross the blood brain barrier. Sitaram et al. (1976) reported REMS-induction by physostigmine infused 30 min after the onset of sleep in man. In a later study (1977) the same group reported a time and dose-dependent effect. In our study we looked at the influence of physostigmine alone, physostigmine combined with methyl-atropine, and carbachol on REMS in the rat.

The effects of atropine sulphate (an anticholinergic agent) on the EEG of normal rats have been described a number of times (e.g. Kramis et al., 1975; Kolb and Whishaw, 1977). The rat is relatively insensitive to atropine; therefore, large doses are required to obtain definitive CNS effects.

We examined the effect of subcutaneous atropine sulphate by release from implanted pellets. This method subjected the rat continuously to a relative constant high dose of the drug. (Fig. 11).

1. Physostigmine

Telemetric pilot study

After several control days, three rats were injected i.p. with 5 mg/kg methyl-atropine. The following day the rats were injected with the same dose of methyl-atropine combined with 50 µg/kg physostigmine salicilate i.p.. On the third day one rat was injected with only physostigmine. Sleep was registered by telemetry for the duration of the experiment (p. 22).

No effect was seen after methyl-atropine alone; however, one rat showed prolonged waking (2 h), and another a total suppression of REMS for 4 h after the combined injection of methyl-atropine and physostigmine. This suppression was not seen after physostigmine alone.

Experiments with physostigmine

Seventeen rats were infused into the jugular vein with several concentrations of physostigmine: 10-100 µg/kg (p. 26). Sleep was recorded via cables (p. 15) during saline infusions and for 60 - 80 min after the drug infusion. Sleep stages were scored (p. 21) and latency and duration of REMS were determined. Infusions of 25 - 100 µg/kg almost invariably caused immediate and prolonged waking periods (10 - 30 min). 10 - 20 µg/kg had no effect on REMS latency.

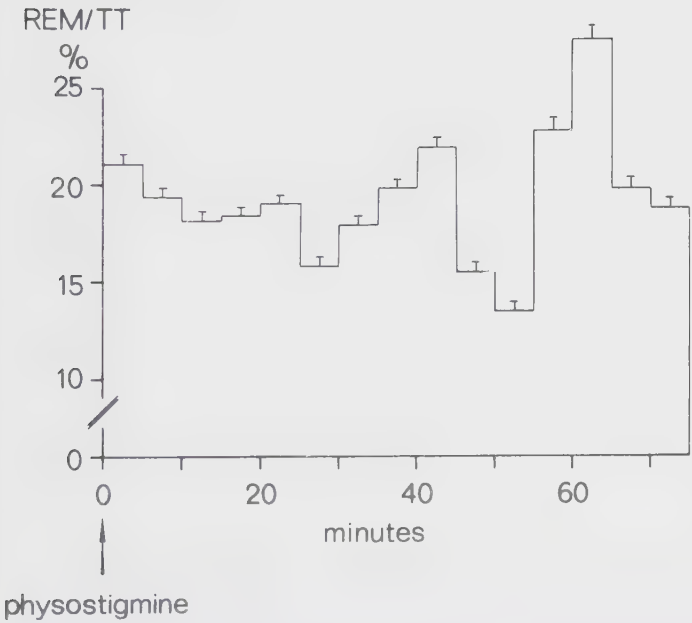
Experiments with methyl-atropine and physostigmine

Eight rats were infused with methyl-atropine 2.5 mg/kg 30 - 60 min before physostigmine 10 - 100 µg/kg was infused into the jugular vein. Infusion of methyl-atropine alone often caused arousals lasting 5 - 10 min, but otherwise had no effect on the distribution of sleep states. The infusion of physostigmine was started after a prolonged uninterrupted sleep period. However, sleep after the combined injections was irregular. NREMS periods were interrupted by frequent awakenings. REMS was neither enhanced nor diminished, and no reduction of REMS latency was observed. (Fig. 26).

Discussion

We were unable to induce REMS in the rat, either after i.p. or i.v. application of physostigmine. Sitaram and coworkers reported (Sitaram et al., 1977), that the length of the induced REM period was in no instance altered, only its latency was shortened. Latency is defined as the duration of NREMS between sleep onset and the first REM period. In the rat sleep cycles

Figure 26:



Effect of intraventricular (jugularis) physostigmine 25 µg/kg infusion (approx. 100 µl/min for 1 - 2 min) on REMS, 30 - 60 min after intraventricular infusion of methyl-atropine 2.5 mg/kg (100 µl/min for 2 - 5 min). Bars represent mean values with SEM of 11 - 18 infusions. TT: Total time.

are too short for applying drugs during a specific sleep state and measuring REMS latency. Furthermore, a great number of controls would be necessary to document that REM periods observed after infusion onset were experimentally induced and did not occur spontaneously.

In human subjects the number of awakenings from physostigmine infusions depended on the sleep stage prevailing at the time of infusion. The same dose which induced REM earlier in the night awakened the subjects, if it was administered in the second NREM period (Sitram et al., 1977).

The predominant arousing effect of physostigmine in our experiments corroborates the suggestion that cholinergic mechanisms are involved not only in the induction of REMS, but also in behavioral arousal.

2. Carbachol: Effect of intraventricular infusions on sleep

Fourteen rats were infused into the lateral ventricle with aCSF-II (p. 36), and with one or two of the following carbachol concentrations: 0.036 µg/min, 0.018 µg/min, 0.009 µg/min and 0.0018 µg/min. The experimental procedure was according to the description in chapter E 3, p. 33 .
A few minutes of infusion of the 3 higher doses invariably elicited prolonged arousal and vigorous feeding. The four rats which were infused with 0.0018 µg/min showed no significant difference in any of the scored vigilance states as compared to aCSF infusion, (i.e. REMS was not induced).
Although Hobson and coworkers reported a state resembling desynchronized sleep (D carb) in cats following intracerebral administration of cholinomimetic drugs, it is not clear if these are true REMS episodes. (Amatruda et al., 1975). Furthermore the intraventricular route of drug administration used in the present study was probably too unspecific, since in addition to the FTG and locus coeruleus cells, carbachol must have affected other cholinoceptive brain structures. Direct injection of carbachol into the pontine brain stem of cats either enhanced or suppressed muscular atonia and cortical synchronization depending on the exact site of application. (Amatruda et al., 1975; Hobson et al., 1975). A study with a more specific location of cholinergic stimulation was realized by Grossmann (1960) who applied cristalline chemicals via a cannula placed in the lateral hypothalamus. "Infusion" of carbachol elicited drinking. By our route of administration carbachol seems to reach the hypothalamus and thus elicit feeding before brain regions are reached which could specifically induce REMS.

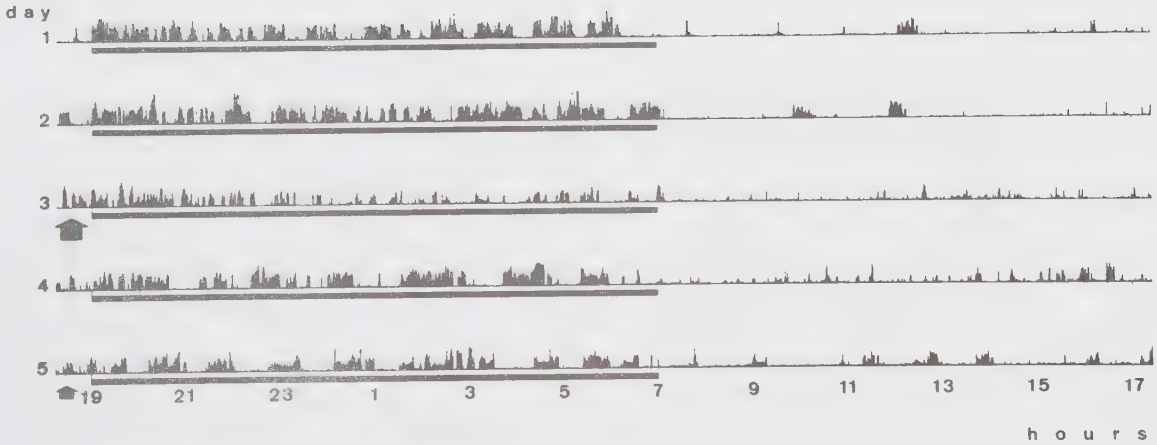
3. Effect of atropine pellets on motor activity

In a pilot study motor activity of two rats implanted with 4 atropine pellets (4 x 30 mg atropine sulphate; method, p. 30) was recorded continuously for 5 days. After two control days the pellets were implanted and removed on the fifth day, on both occasions at the end of the light-phase. The motor activity pattern was unequally affected during the dark and light phases. Dark time activity on day three was markedly reduced, and showed a succession of relatively long activity bouts and rest periods on the following night. This effect persisted on the night after removal of the pellet, which could be either a persistant atropine effect or a recovery of sleep lost during the two days under atropine influence. During the light-phase motor activity was enhanced, yet the rare prolonged activity bouts were absent. They reappeared after removal of the pellets on day 5 (Fig. 27).

4. Effect of atropine pellets on sleep

Two rats were implanted with 4 pellets (4 x 30 mg atropine sulphate) after they had been adapted to cable recording. EEG was recorded daily with exception of day 6, for 2 - 4 h and sleep states were scored visually (p. 21 , Table 6). Day 1 and 2 served as controls; at 18:00 h on day 2 the pellets were implanted, and registration continued for 5 days.
Atropine had no effect on total sleep time as compared to control days, whereas REMS was slightly reduced on the first day after the implantation. This was not surprising, since atropine (1 - 2 mg/kg) was the first drug found to suppress REMS selectively. (Jouvet, 1961 in cats; Khazan and Sawyer, 1964 in rabbits; Toyoda et al., 1966 in man). The EEG during REMS showed an irregular pattern with frequent bursts of high frequency and amplitude waves, which might be associated with the well documented effects of atropine sulphate on the hippocampal EEG during REM bursts (Kolb and Whishaw, 1977).

Figure 27:



Motor activity of a rat recorded continuously for 5 days and plotted for consecutive 40-s periods (arbitrary units). Dark-phase is indicated by bar below records. Larger arrow on day three indicates the time of implantation of four pellets, each containing 30 mg atropine sulphate. Smaller arrow on day 5 indicates the time of pellet removal. Time of day is indicated on abscissa.

Table 6:

	Distribution of vigilance states (% of recording time)				
		NREMS		REMS	
	Day	Rat 1	Rat 2	Rat 1	Rat 2
Control	1	63.83	61.83	16.61	12.06
	2	63.14	66.23	12.57	10.62
Atropine 120 mg	3	66.71	70.25	9.08	7.67
	4	69.07	67.39	10.02	9.31
	5	61.20	65.08	12.44	10.69
	6	-	-	-	-
	7	66.40	59.36	13.29	11.21

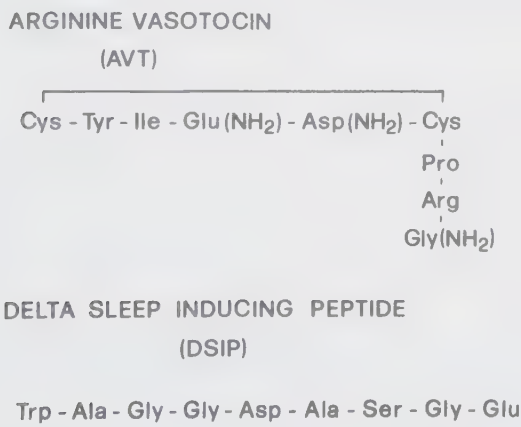
Sleep parameters for 2 - 4 h recording sessions on two control days and on five days after the implantation of subcutaneous pellets containing 120 mg atropine sulphate. NREMS (nonREM sleep).

V PEPTIDES AND HUMORAL SLEEP FACTORS

A Peptides

The two nonapeptides, delta sleep inducing peptide (DSIP) and arginine vasotocin (AVT) (Fig. 28) have both been reported to promote sleep. DSIP was isolated and identified as the active sleep-enhancing principle in the ultradyalisate of venous blood of rabbits that had been subjected to electrical stimulation in the thalamus (Monnier and Hösli, 1964; Schönenberger et al., 1977; 1978). When this dyalisate was administered to recipient rabbits they fell asleep and their NREMS showed a predominant activity in the delta-band (1 - 4 Hz). In 1977 the active principle was isolated and named Delta-Sleep-Inducing Peptide (DSIP). (Schönenberger et al., 1977).

Figure 28:



Structure of arginine vasotocin (AVT) and Delta Sleep Inducing Peptide (DSIP).

AVT bears a close structural relationship to both vasopressin and oxytocin from each of which it differs only by a single amino acid. It has long been known to occur in the neurohypophysis of lower vertebrates, but its presence in the pineal gland and ependymal cells of the pineal recess in mammals has been reported only recently (Pavel, 1978; Pavel et al., 1978). Pavel and coworkers (1977b) have described a sleep-inducing action of AVT when it is injected in minute amounts (10^{-6}pg $\approx$ 10^{-21}mol, corresponding to 600 molecules) into the third ventricle of the cat. In recent papers it has been proposed that REM sleep is related to exogenous or endogenous AVT (Coculescu et al., 1979; Pavel et al., 1980).

In the following experiments we investigated the effect of the two peptides on sleep in the rat. The presence of DSIP-like immunoreactive material in rat brain (Kastin et al., 1978) may suggest a functional role of the peptide. A mild sleep-enhancing action has been recently described for DSIP in this species (Kafi et al., 1979). However, there are indications that a potential stress reducing effect of DSIP may have been interpreted as a specific sleep-inducing action; therefore we habituated our rats very carefully to the recording chamber, to handling and to injections.

AVT has been recently reported to be ineffective after systemic administration in the rat at doses that have been reported to be effective in the cat (5×10^{-10}mol/kg) (Mendelson et al., 1980). However, this substance seems to exhibit a potent biological activity in this species, since exceedingly small doses of AVT (10^{-4}pg $\approx$ 10^{-19}mol) administered into the third ventricle were reported to decrease the plasma level of luteinizing hormone (LH), and increase the hypothalamic serotonin content (Pavel et al., 1979).

In order to further examine this difference between the cat and the rat, we conducted our experiments with rats choosing the intraventricular route of administration.

1. Experiments with Delta Sleep Inducing Peptide (DSIP)

Effect of DSIP on motor activity

All manipulations of the 14 animals were carried out between 17:30 and 18:00 h. After 7 days of habituation to the experimental chamber, the animals were handled for a few minutes every day for 4 days. The next five days all animals received an i.p. injection of saline. The following day, 7 rats were injected i.p. with 40 nmol/kg and 7 with 80 nmol/kg DSIP freshly dissolved in saline.
The first group then received saline on day 10, again 40 nmol/kg on day 11 and a last saline injection on day 12. The second group was injected with saline only on the last three days (Table 7).
Another group of 6 rats was registered for three undisturbed control days, on day four they were injected with 160 nmol/kg DSIP, the last day serving as an uninjected control (Table 7).
There was no effect of DSIP 40 or 80 nmol/kg on motor activity compared to control injection of NaCl (Fig. 29).
An analysis of the first four hours after DSIP administration compared to the four hours following the last saline injection before DSIP shows no difference for 40 nmol/kg and a significant enhancement of motor activity after 80 nmol/kg (Fig. 30). ($P < 0.05$, two-sided paired t-test).
Motor activity during the dark phase or in the 24 hours after 160 nmol/kg DSIP was not significantly different from the mean value of the three control days. There was a significant reduction in the dark phase one day ($p < 0.02$) and two days after DSIP administration ($p < 0.05$, two-sided paired t-test).

Fig. 29: Effect of DSIP on motor activity in 2 groups of 7 rats which were recorded for 11 days (left column) or 13 days (right column). The 12-h dark period is indicated by a bar under the abscissa. The plots represent mean values for successive 15-min periods (arbitrary units; baseline corresponds to zero activity). All manipulations were made immediately before dark onset.
HAND.: handling (4 days); NaCl: i.p. injection of saline (5 days); DSIP: i.p. injection of 40 nmol/kg (left) or 80 nmol/kg (right); NaCl: post-drug control injection of saline (1 or 3 days).

Figure 29:

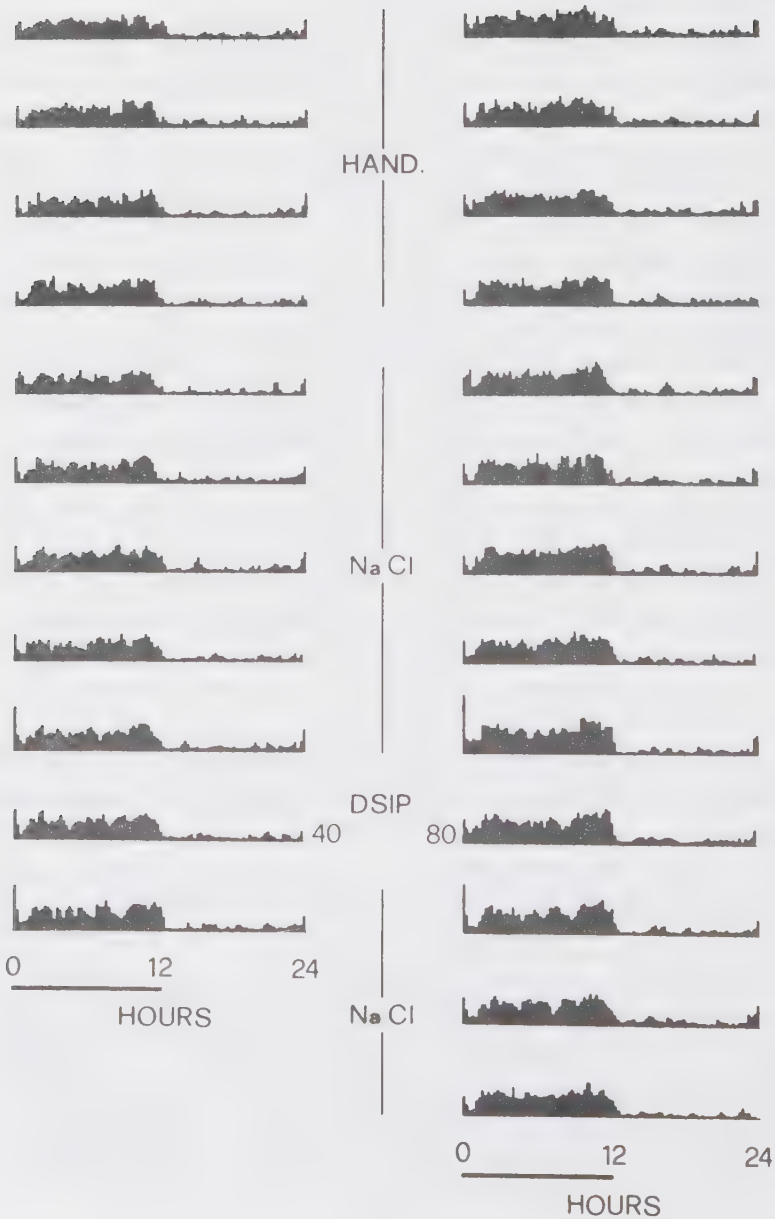
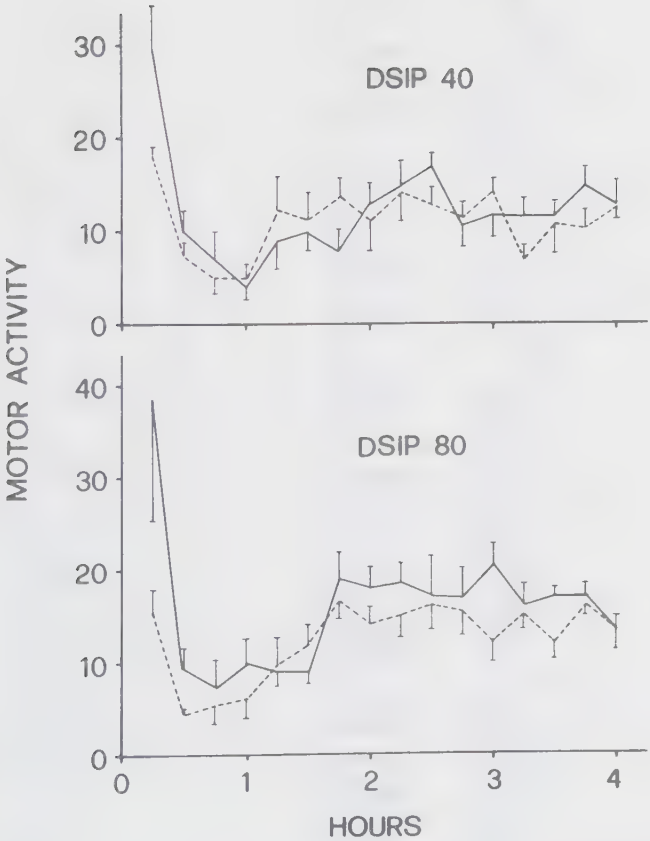
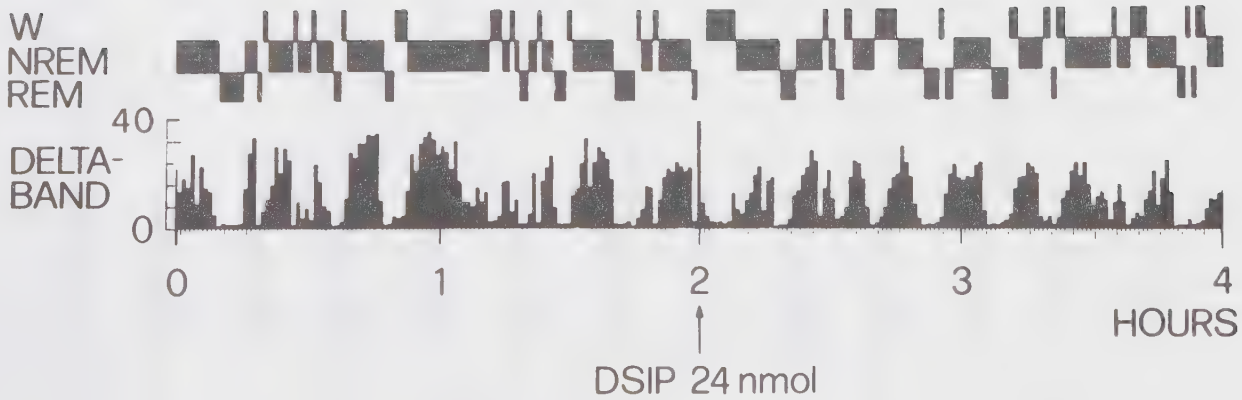


Figure 30:



Effect of DSIP on motor activity in 2 groups of 7 rats. The curves show the values for the 4-h post-injection period. Interrupted curves: i.p. injection of saline on the day before DSIP administration; continuous curves: i.p. injection of DSIP 40 nmol/kg (top) or 80 nmol/kg (bottom). Mean values with SEM plotted in arbitrary units for successive 15-min periods.

Figure 31:



Vigilance states (W: waking; NREM: nonREM sleep; REM: REM-sleep) and spectral power density ($(\mu V_{RMS})^2$/0.25 Hz) of the EEG in the delta-band (1.25 - 4.0 Hz) before and after injection of DSIP (24 nmol; arrow) into the third ventricle of a rat. The vigilance states and power density values are plotted for successive 1-min periods (peak at injection time is an artifact due to handling).

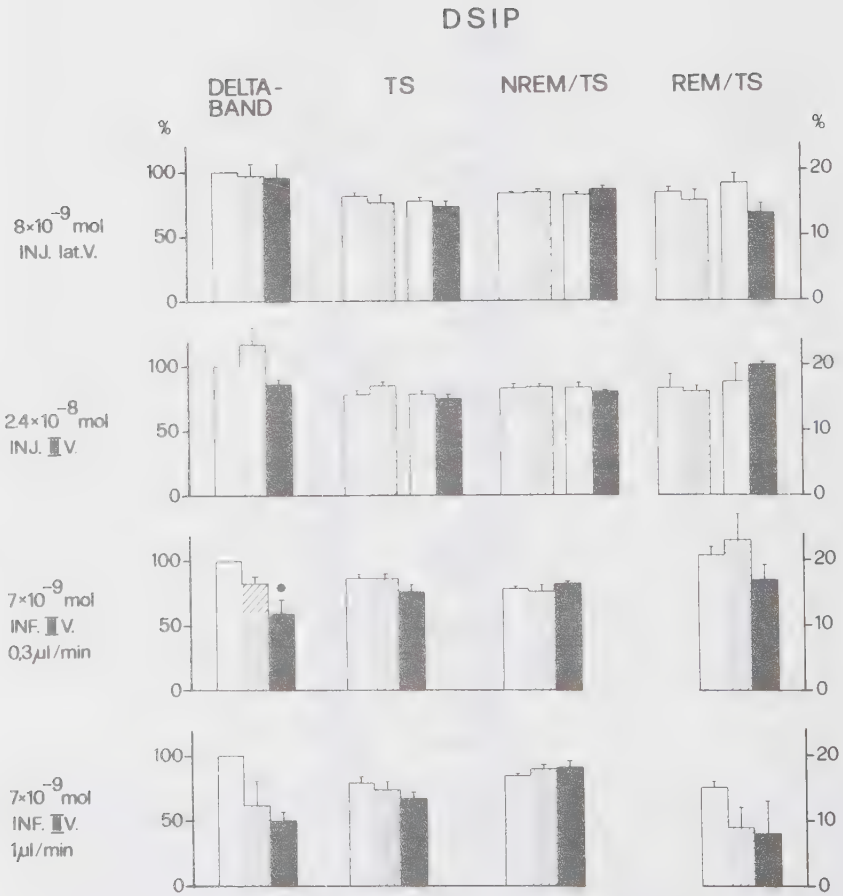
This finding suggests a protracted activity reducing effect of DSIP 160 nmol/kg which was only seen after an activation in the dark phase immediately following the injection. To further test this possibility, 15 rats were habituated to the experimental chamber for 2 days, the next two days served as controls (day 1 and 2) and on day 3, 8 rats received 160 nmol/kg DSIP i.p. and 7 rats were injected with saline. Registration of motor activity was continued for 4 days after injection. (Table 7).
No tendency towards reduction of motor activity was seen either on the first night after DSIP injection or in the following 3 control dark periods without injection. A slight enhancement of motor activity throughout the experiment was seen also in the saline controls.

Effect of DSIP on sleep

Four rats were injected in the third ventricle with 24 nmol DSIP and four in the lateral ventricle with 8 nmol DSIP. Four rats were infused in the third ventricle for 2 hours with a total amount of 7 nmol DSIP at the infusion rate of ca 1 µl/min and 0.25 µl/min. Injections of 24 and 8 nmol DSIP caused no significant reduction in the delta band compared to saline injections (Figs. 31 and 32).
A 2 h infusion of DSIP 7×10^{-4} mol at the infusion rate of 0.3 µl/min caused a significant reduction ($p < 0.05$) in the delta band (Fig. 32).

Fig. 32: Effect of intraventricular injection (INJ.) or infusion (INF.) of DSIP into the lateral (lat.V.) or third ventricle (III V.) on sleep. White bars represent pre-injection or pre-infusion control values, hatched bars values after saline administration, and dark bars values after DSIP (dose and infusion rate indicated on left). Delta-band: Spectral power per NREM-sleep expressed as percentage of control (n = 3 - 4; see p. 22). TS: Total sleep expressed as percentage of recording time; NREM-sleep and REM-sleep are expressed relative to TS (n = 4). Right ordinate applies only to REM/TS. Mean values with SEM. Asterisk: significant difference from saline value ($p < 0.05$; 2-sided paired t-test).

Figure 32:


DSIP
DELTA-BAND
TS
NREM/TS
REM/TS
%
%
8×10⁻⁹ mol INJ. lat.V.
2.4×10⁻⁸ mol INJ. IV.
7×10⁻⁹ mol INF. IV. 0,3µl/min
7×10⁻⁹ mol INF. IV. 1µl/min
100
50
0
20
10
0

There were no significant differences in total sleep, NREMS and REMS between saline and DSIP injections or infusions at any of the concentrations used.

2. Experiments with arginine vasotocin (AVT)

Effect of AVT on behavior

Six rats were infused with 1.5×10^{-2}pg and nine rats with 1 pg AVT in the lateral ventricle. (Pilot studies in the lateral ventricle included concentrations from 1.5×10^{-2} - 4.5×10^{3} pg, and i.p. injections from 1 µg - 10 ng/kg).
Behavior was scored by observing the animal during 15 min infusion and for another 15 min after the infusion. The testing occurred on consecutive days at the same time of day (17- 19h). All rats received a control saline infusion. The occurrence of three criteria: (1) activity, (2) inactive waking, and (3) sedation or sleep within 30-sec epochs was scored for 5 min bins and expressed as a percentage value.
No evidence for an activity reducing or a sleep enhancing effect of AVT as compared to saline was found (Fig. 33).

Effect of AVT on sleep

Two groups of four rats each were injected in the third ventricle with AVT 1 pg and 10^{-2}pg and with 10^{-2} and 10^{-4}pg, respectively. Pilot studies included infusions from 10^{-4} to 10^{2}pg AVT. (10^{-15}, 10^{-17} and 10^{-19} mol AVT .)
A significant reduction was found in the delta band compared to saline control after an injection of AVT 10^{-17} mol ($p < 0.05$, two-sided paired t-test). Fig. 34.
There were no significant differences in total sleep, NREMS and REMS between saline and AVT administration at any of the concentrations used.

	Days	INDIVIDUAL DAYS Tot	D	L	MEAN OVER DAYS Tot	D	L
Handling	1	7.44 (0.50)	11.42 (0.72)	3.28 (0.39)	7.75 (0.40) (0.21)	12.12 (0.61) (0.46)	3.19 (0.33) (0.15)
	2	8.07 (0.67)	12.44 (0.90)	3.51 (0.60)			
	3	7.35 (0.46)	11.35 (0.60)	3.16 (0.43)			
	4	8.15 (0.66)	13.26 (1.07)	2.81 (0.29)			
NaCl	5	7.21 (0.20)	11.38 (0.24)	2.85 (0.27)	7.79 (0.34) (0.19)	12.26 (0.45) (0.24)	3.30 (0.21) (0.17)
	6	8.02 (0.42)	12.34 (0.73)	3.51 (0.10)			
	7	8.11 (0.32)	12.36 (0.48)	3.68 (0.32)			
	8	7.73 (0.57)	12.31 (0.97)	2.95 (0.26)			
	9	8.31 (0.48)	12.89 (0.58)	3.53 (0.39)			
DSIP	10	8.19 (0.38)	13.14 (0.70)	3.02 (0.15)			
NaCl[i]	11	8.90 (0.45)	14.01 (0.78)	3.57 (0.26)			
	12	c	d	e			
	13	f	g	h			

	Days	INDIVIDUAL DAYS Tot	D	L	MEAN OVER DAYS Tot	D	L
Handling	1	8.97 (0.59)	13.99 (0.82)	3.73 (0.52)	8.99 (0.56) (0.08)	13.57 (0.89) (0.21)	3.84 (0.47) (0.07)
	2	8.95 (0.78)	13.71 (1.06)	4.00 (0.67)			
	3	8.82 (0.48)	13.52 (0.71)	3.91 (0.37)			
	4	9.22 (0.68)	14.49 (0.97)	3.72 (0.47)			
NaCl	5	8.89 (0.50)	14.26 (0.79)	3.28 (0.43)	9.26 (0.62) (0.22)	14.91 (0.96) (0.42)	3.37 (0.40) (0.07)
	6	9.05 (0.66)	14.43 (1.12)	3.43 (0.36)			
	7	9.43 (0.84)	15.02 (1.19)	3.59 (0.63)			
	8	8.90 (0.57)	14.35 (0.87)	3.21 (0.38)			
	9	10.06 (0.72)	16.49 (1.24)	3.34 (0.50)			
DSIP	10	9.59 (0.68)	15.36 (0.92)	3.57 (0.58)			
NaCl[i]	11	9.08 (0.85)	14.62 (1.23)	3.30 (0.52)	8.75 (0.62) (0.18)	14.01 (0.94) (0.32)	3.28 (0.36) (0.23)
	12	8.71 (0.62)	13.55 (0.84)	3.66 (0.46)			
	13	8.47 (0.53)	13.85 (0.92)	2.87 (0.36)			

D S I P 160 n m o l / k g

	Days	INDIVIDUAL DAYS Tot	D	L	MEAN OVER DAYS Tot	D	L
Control	1	7.93 (0.40)	13.23 (0.61)	2.39 (0.35)	8.29 (0.43) (0.18)	13.91 (0.64) (0.34)	2.43 (0.33) (0.05)
	2	8.49 (0.55)	14.20 (0.86)	2.52 (0.39)			
	3	8.45 (0.40)	14.28 (0.58)	2.36 (0.29)			
DSIP	4	7.89 (0.45)	13.06 (0.67)	2.50 (0.23)			
Control	5	8.13 (0.31)	13.19[a] (0.51)	2.86 (0.17)			
	6		12.91[b] (0.49)				

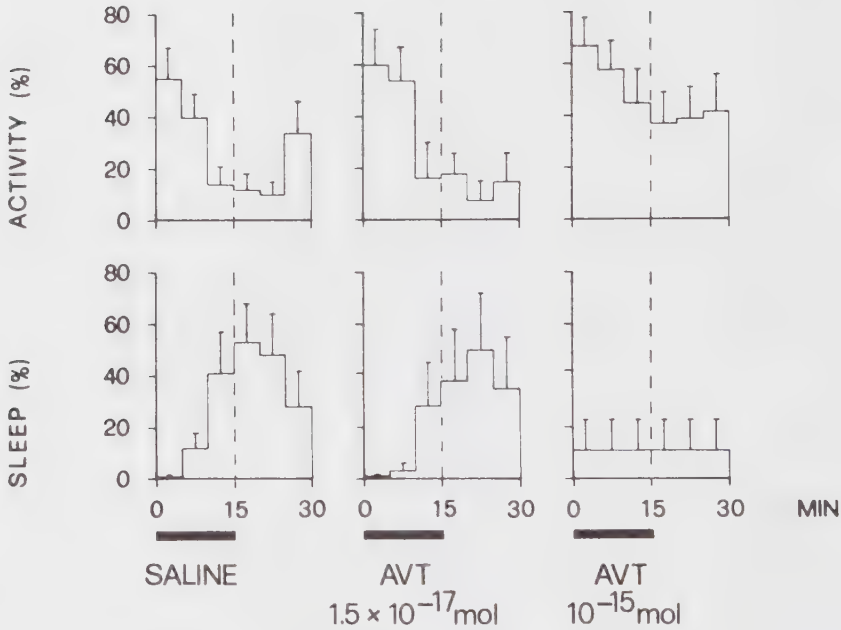
		DSIP 160 nmol/kg						NaCl					
		INDIVIDUAL DAYS			MEAN OVER DAYS			INDIVIDUAL DAYS			MEAN OVER DAYS		
	Days	Tot	D	L	Tot	D	L	Days	Tot	D	L	Tot	D	L
Control	1	8.10 (0.87)	13.12 (1.29)	2.86 (0.55)	8.20 (0.91) (0.10)	13.35 (1.33) (0.23)	2.83 (0.56) (0.03)	Control 1	8.85 (0.29)	14.39 (0.61)	3.08 (0.08)	9.09 (0.30) (0.24)	14.76 (0.65) (0.37)	3.17 (0.12) (0.09)
	2	8.30 (0.97)	13.57 (1.42)	2.80 (0.57)				2	9.32 (0.34)	15.13 (0.78)	3.25 (0.21)			
DSIP	3	9.26 (1.09)	15.40 (1.74)	2.86 (0.50)				NaCl 3	9.77 (0.83)	15.40 (1.24)	3.91 (0.52)			
Control	4	8.73 (0.90)	14.42 (1.36)	2.79 (0.50)	9.29 (0.81) (0.50)	14.95 (1.26) (0.37)	3.38 (0.41) (0.66)	Control 4	10.19 (1.01)	16.30 (1.61)	3.82 (0.65)	10.54 (0.97) (0.36)	16.57 (1.51) (0.25)	4.25 (0.58) (0.48)
	5	8.84 (0.85)	14.77 (1.33)	2.66 (0.48)				5	10.16 (0.95)	16.34 (1.49)	3.71 (0.62)			
	6	10.29 (0.75)	15.66 (1.22)	4.69 (0.35)				6	11.26 (0.96)	17.06 (1.47)	5.21 (0.53)			

Effect of DSIP on motor activity. All injections were administered intraperitoneally before dark-onset. Mean values (SEM in parenthesis) over animals for individual days, or over animals and days (mean over days, where upper row of parenthesis indicates SEM for means over animals and lower row SEM for means over days). Control values without injection for DSIP 160 nmol/kg (n = 6, other doses n = 7). Motor activity in arbitrary units.

Significant difference from the mean value of NaCl control days [a] $p < 0.02$, [b] $p < 0.05$ (two-sided paired t-test).

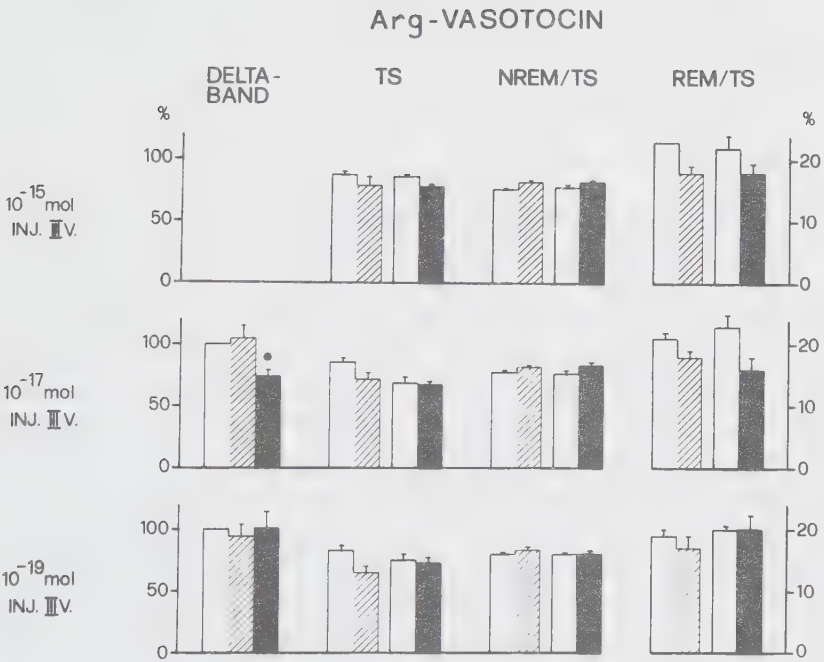
[i] The group treated with DSIP 40 nmol/kg on day 10 received the same dose a second time on day 12 and NaCl on day 13. The values with SEM are: [c] 8.33 (0.36), [d] 12.94 (0.70), [e] 3.52 (0.14), [f] 7.82 (0.37), [g] 12.28 (0.54), [h] 3.16 (0.25).

Figure 33:



Effect of arginine vasotocin (AVT) on visually scored activity and sleep. The 15-min infusion (3 µl/min) into the lateral ventricle is indicated by dark bar under abscissa, and delimited from the post-infusion period by an interrupted vertical line. Histogram indicates for 5-min bins percentage of 30-s periods in which activity (top) and sleep (bottom) was scored (mean values with SEM; n = 6 - 9).

Figure 34:



Effect of intraventricular injection of arginine-vasotocin (AVT) into the third ventricle on sleep. Mean values with SEM (top and bottom rows: n = 4; middle row: n = 8). Asterisk: significant difference from saline value ($p < 0.05$; 2-sided paired t-test). See legend of Fig. 32 for further details.

Effect of AVT on hypothalamic serotonin

Six saline injected controls had 5.27 + 0.29 nMol/g serotonin in the hypothalamus, six rats injected in the third ventricle with 3 µl containing 10^{-2} pg AVT, 5.33 + 0.29 nMol/g. No significant difference.

3. Discussion

In the present study we were unable to find evidence for an immediate sleep-promoting action of DSIP or AVT in the rat. In view of the discrepancy of these negative results with several previous reports, it was important to ascertain that we were using the pure peptides and that they remained intact in the solutions we administered. The results of the chemical and biological assays (p. 51) leave little doubt that this has been the case. Are there other arguments that could be advanced to account for the lack of positive findings? It could be argued that we failed to detect the minor soporific effect demonstrated by Kafi and coworkers (1979). However, one would expect that the spectral analysis of the EEG would reveal in this case an increased spectral power in the delta band, an effect that was reported to be the hallmark of the DSIP action in the rabbit (Schoenenberger et al., 1977; 1978), and constitutes a very sensitive indicator of increased 'sleep-pressure' in the rat (Borbély and Neuhaus, 1979). Yet, if anything, the power in the delta-band was reduced by both DSIP (Fig. 32) and AVT (Fig. 34). Did we use an ineffective dosage range? There are indications that doses of DSIP exceeding the optimal range may lose their sleep-promoting effect in the cat (Polc et al., 1978). However, our doses used for systemic administration (40 - 160 nmol/kg) were similar to the effective doses that have been reported for the rat (30 - 80 nmol/kg; Kafi et al., 1979), cat (30 nmol/kg; Polc et al., 1978) and rabbit (30 nmol/kg; Monnier et al., 1977), although the intraperitoneal route has not been used previously. The amount of DSIP administered into the ventricle (7 - 24 nmol/

animal) was higher than the effective dose in the rabbit (nmol/kg; Schoenenberger et al., 1977). However, with the lowest infusion rate (0.3 µl/min) and a total amount of 7 nmol administered over approximately 2 hours, the effective intraventricular concentration is bound to remain low. The dosage range of AVT was similar to that used by Pavel and coworkers (1977a; 1979) in the cat and rat. In conclusion, we were administering the pure and intact peptides in doses which have yielded positive results in previous experiments, and our analysis procedures should have been sensitive enough to detect even a minor increase in SWS or other vigilance states.

A close comparison of the published data on DSIP and AVT reveals species-differences which may be relevant for the interpretation of the present findings. Thus, while in the rabbit DSIP enhances specifically 'delta sleep' (Schoenenberger et al., 1977; 1978), its main effect in the cat seems to be the enhancement of REMS (Polc et al., 1978). In the rat, the action of DSIP on sleep states remains largely unspecified (Kafi et al., 1979). A similar situation prevails for AVT which enhances SWS and suppresses REMS in the cat (Pavel et al., 1977a), but appears to promote REMS in man (Coculescu et al., 1979). These inconsistent effects of DSIP and AVT on sleep states of different species stand in contrast with the action of sleep factor S which enhances SWS in both the rat and rabbit, and thereby mimics the effect of sleep deprivation (Fencl et al., 1971; Pappenheimer et al., 1975). Could it be that neither DSIP nor AVT represent <u>specific</u> sleep-promoting substances, and that their effects on sleep constitute some indirect or secondary action? Several arguments can be advanced in support of this hypothesis: First, an immediate stimulating effect on motor activity was seen in the first 4 hours after a dose of 80 nmol/kg (Fig. 30). Thus an opposite effect on activity seemed to be present in the rat contrary to a short-term enhancement of sleep. Second, a clear reduction of the power in the delta band was seen after AVT at an intraventricular dose of 10^{-17}mol

which was somewhat higher than the dose reported to decrease the plasma level of LH in rat (10^{-19} - 10^{-20}mol; Pavel et al., 1979) and of cortisol in cat (Pavel et al., 1977b). Although we could not confirm the AVT-induced increase in hypothalamic serotonin (Pavel et al., 1977b; 1979) in the unanestetized rat, we were clearly dealing with a peptide with extremely potent endocrine action. Changes in sleep could be therefore secondary to the hormonal changes. Regardless of the mechanisms involved, it should be emphasized that the potency of AVT in influencing the EEG is unique, and exceeds by far any other known substance. Third, there is the possibility that the sleep-enhancing effect of DSIP may be related to a tranquilizing or stress-reducing action. Thus DSIP was found to counteract morphine-induced insomnia in the cat, an effect that was described in the same study also for various benzodiazepines (Scherschlicht et al., 1979). Moreover, a stress-reducing action may account for the sleep-enhancing effect of DSIP reported by Kafi and coworkers (1979) for the rat, since these authors administered the drug by an injection into the tail vein which involves immobilization and pain. Their control level of sleep was also clearly lower than in our experiments. By the same token, our attempts to minimize stress may have prevented any observable effect on sleep.

In view of the possibility that DSIP and AVT may influence sleep by some nonspecific action, we attempt to specify the requirements for a <u>specific</u> sleep-promoting substance by proposing the following criteria: (1) Sleep should be reliably and distinctly induced and/or maintained; (2) within a specific dosage range a dose-effect relationship should prevail; (3) in different species, the action of the substance should be similar with respect to timecourse and effect on sleep states; (4) the substance should be present in the organism; (5) endogenous changes (in concentration, turnover, etc.) should occur in association with the physiological sleep-waking cycle; (6) the substance should be chemically identified. Only the criteria

(4) and (6) seem to apply unequivocally to DSIP and AVT. We conclude therefore on the basis of the present results and the published data, that the evidence is insufficient to attribute a specific sleep-promoting action to either of these peptides. Of the agents which have been proposed so far as a 'sleep substance' or 'sleep factor', Factor S of Pappenheimer and coworkers seems to come closest to a specific sleep-promoting substance, since on the basis of the published data it may satisfy all but the last criterion (Fencl et al., 1971; Pappenheimer et al., 1975; Krueger et al., 1978). The brain extract of sleep-deprived rats which has been studied by Nagasaki and coworkers (1974) exhibits properties similar to Factor S although the physiological data are less extensive.

B Humoral sleep factors

The first investigators who looked for a sleep inducing agent (Piéron, 1913) kept dogs awake for 10 days by exercising them. CSF from these animals was reported to have induced sleep in recipient dogs. The substance hypothetically involved was called hypnotoxin. Fifty years later Pappenheimer and coworkers reported that CSF from sleep deprived goats infused into the ventricle of rats' brains induced sleep. (Pappenheimer et al., 1975).
The increasing evidence that during sleep deprivation a factor accumulates in the CSF or brain tissue which induces sleep in recipients (Nagasaki et al., 1974; Pappenheimer et al., 1975) led to our following experiment.

1. CSF from sleep-deprived rats

Motor activity

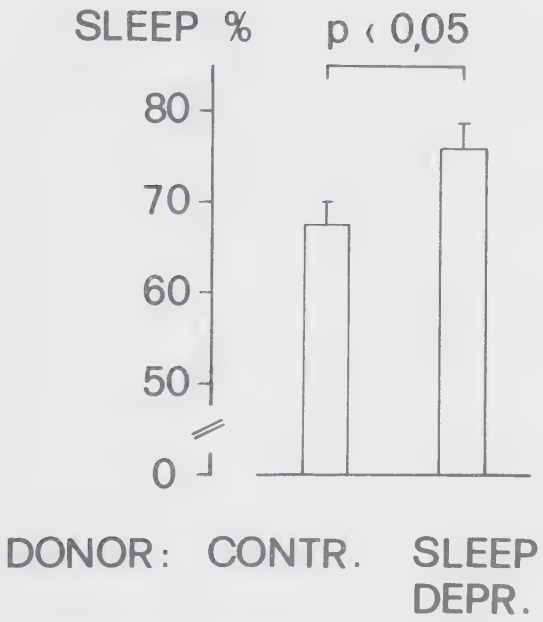
Cerebrospinal fluid was collected from rats which had been sleep deprived for 24 h (depr.CSF) (p. 14) or from normal rats (CSF, control).

Before experiment on p. 38 was concluded, six rats received an additional infusion of depr.CSF (3 µl/min) and motor activity was measured for 24 h after the infusion (p. 13). There was no significant difference in motor activity after depr.CSF infusion compared to CSF during the 12 h dark following both infusions.

Sleep

Six rats which had been previously adapted to cable recordings received each two infusions, CSF and depr.CSF on consecutive days. The infusions lasted ca 20 min (infusion rate 3 µl/min). Sleep was recorded in the 12 h light phase, during infusions and for at least three hours after every infusion. The distribution of the vigilance states W, NREMS and REMS (p. 21) 1 h, 2 h and 3 h after CSF or depr. CSF were compared. Fig. 35 illustrates the effect of the two infusions on total sleep time. There was slightly, but significantly, more TS 2 and 3 hours after depr.CSF compared to CSF ($p < 0.05$). NREMS was also significantly enhanced 2 and 3 h after depr.CSF ($p < 0.025$), whereas the percentage of REMS was not significantly altered.
These preliminary results indicate that the sleep deprivation method may be adequate to obtain "hypnotoxin" in the CSF which can then enhance sleep in recipient rats. Experiments in progress will show if there is more SWS after depr.CSF than after CSF. The weak and delayed effect (2 - 3 h after infusion) obtained in the recipient rats may be due to a very small concentration of the active principle. To achieve clearer effects, longer infusions with a smaller infusion rate of a concentrated or even fractioned CSF should be tested. In addition it should be kept in mind, that the potential sleep enhancing substance was tested in rats during the light phase, that is during a time when the animals sleep optimally under normal circumstances. The rat is not a suitable species to test sleep latency for instance after cerebroventricular injection of a concentrated principle since there is a wide range of individual reactions to any kind of disturbance during day-time hours.

Figure 35:



Sleep-enhancing action of CSF from sleep deprived donor rats (sleep depr.). Bars represent mean percentage of sleep (with SEM) during hour 2 and 3 after a 20 min infusion of natural cerebro-spinal fluid (CSF, control) or of CSF taken from sleep deprived donor rats into the lateral ventricle of recipient rats. Differences between TS values for control and for sleep depr. were significant, $p < 0.05$ (one-sided paired t-test, $n = 6$).

VI CONCLUDING REMARKS

Our interest in this study has focused on sleep and its regulation. Physiological and pharmacological methods were used to induce sleep or specific sleep states.
Sleep can be induced by environmental agents such as light and temperature. Light, apart of the daily light-dark cycle, is not only a synchronizer of many circadian rhythms, but has also a strong sleep-inducing effect. In this respect light, the most potent environmental stimulus for sleep induction, is as powerful as a drug, and thus may be profitably used as an experimental tool for studying the vigilance states and the neurochemical processes underlying their regulation (Borbély, 1978).

In this work three agents considered to have specific sleep inducing properties were studied: Delta Sleep Inducing Peptide (DSIP), arginine vasotocin (AVT) and cerebrospinal fluid from sleep deprived animals. The results in the rat do not support an immediate sleep-promoting action of DSIP or AVT. The inconsistent effect of these substances on sleep states in different species may be caused by non-specific influences on sleep. On the basis of the results in this study it is concluded that there is not enough evidence to attribute a sleep-inducing effect to either of the two peptides. However, the preliminary study with CSF from sleep deprived rats has shown promising results, adding to the increasing evidence that during sleep deprivation a factor is accumulated with specific sleep-inducing properties (Pappenheimer et al., 1975).

The induction of specific sleep states can be accomplished by short light-dark (LD) cycles as well as by various drugs. Short LD-cycles selectively induce REMS during the dark period. In this study it has been shown that, in addition to the rat, this effect applies to at least one further species. During short LD-cycles REMS episodes in the golden hamster were redistributed

so that they occurred mainly during the light period. The mechanisms involved in this powerful action of an environmental agent have not been identified. It has been shown (chapter III) that the effect is not limited to the highly light sensitive albino rat, but is present also in a non-albino strain. The short latency of REMS induction by dark periods indicates that a neuronal mechanism or a rapid neurochemical process is involved. The possibility that a pituitary hormone is responsible for the rapid REMS induction, has been eliminated in this study, since hypophysectomized rats clearly showed the effect.

There is evidence that the cholinergic system is involved in REMS induction (Sitaram et al., 1977, in humans; Hobson et al., 1975, in cats). The findings in the rat with physostigmine and carbachol failed to confirm these results. With the intraventricular approach used in the present study, various unspecific pathways may have been activated, thus masking specific cholinergic effects. REMS induction by intravenous application of cholinergic substances in humans might be mediated by rather specific cholinergic pathways. It is well known, that light does not only affect behavioral parameters, but may also modify specific neuroendocrine processes in the brain. Brief episodes of light induce a rapid and prominent decrease in activity of pineal N-acetyltransferase, (Axelrod, 1974). Thus it is tempting to speculate that light-dark transitions might induce REMS in the rat by activating a specific neurochemical process involving cholinergic pathways. A study of the relations between ACh turnover, sleep, and short LD-cycles may yield valuable information on the role of the cholinergic system in the sleep of the rat.

A further aspect to be considered is a possible NREMS facilitating effect of short light periods which possibly involves the serotonergic system. Cortical levels of 5-HT and 5-HIAA were increased within 15 min after onset of light (Hanselmann and Borbély, 1975), indicating that the enhancement of 5-HT turnover

may underlie the sleep-inducing effect of light. Additional evidence for a close relationship between 5-HT and sleep in the rat derives from the results that brain levels of 5-HT and 5-HIAA measured during recovery sleep after 24 h sleep deprivation were increased (Borbély et al., 1980).
Finally, it has been shown (chapter IV) that 24 h after PCPA administration sleep is disrupted, and forebrain 5-HT levels are low. TRP, by raising the cerebral 5-HT level, first restored SWS, the fraction of NREMS with high delta activity (1.25 - 4.0 Hz).

It became clear in this study that if NREMS is viewed as a homogenous sleep state, subtle influences on sleep may be overlooked. A spectral analysis of the EEG was needed to reveal shifts in the EEG frequency within NREMS after PCPA and TRP application. Furthermore, with this method FU 29-245 was shown to have not only a NREMS enhancing effect, but also a selective effect on the delta band, whose power was significantly increased. In view of the unclear activating effect of intracerebral 5-HT infusions and the negative results with intravenous LSD application in this study, the strong positive effect of FU 29-245, a serotonin antagonist, is astonishing. Biochemical studies with other agents, like methiotepin and methysergide that are known to have antiserotonergic properties from behavioral studies, have shown that both substances may not act on the same postsynaptic serotonin receptors in the brain (Bourgoin et al., 1978). The 5-HT antagonistic properties ascribed to FU 29-245 have been documented by behavioral studies (Spiegel, 1979). Further biochemical studies are needed to define its precise mechanism of action on 5-HT receptors in the brain, which may possibly account for the sleep enhancing property of the drug.

VII SUMMARY

- Mechanisms underlying sleep and motor activity were investigated in the rat. The EEG and EMG were recorded either via cable connections or by telemetry.
- To avoid disruption of sleep by the usual drug administration procedures, methods were developed which permitted intravenous or cerebroventricular infusion in the sleeping animal.
- Sleep and behavior of the rat were recorded during infusion into the lateral ventricle of artificial cerebrospinal fluid (aCSF) containing normal or excess concentrations of calcium and magnesium. Infusion of aCSF with a normal concentration of Ca did not alter the amount of total sleep, but decreased REM-sleep (REMS) by more than 50 %. Depression of REMS did not occur when the concentration of Ca in the infusate was increased. Possibly Ca acts upon REMS-generating structures in the brainstem.
- Infusions of aCSF containing three times the usual Mg concentration of 2 mM did not affect sleep. Higher concentrations of Mg or Ca induced vigorous and prolonged feeding.
- Rats were exposed to short light-dark cycles to study the redistribution of REMS. REMS predominated during the short dark periods in the albino strain, and to a lesser extent in a pigmented strain. Hypophysectomy did not affect the predominance of REMS during the dark periods. NonREM sleep (NREMS) showed practically an equal distribution between light and dark periods. Thus neither the albino condition nor the presence of the pituitary gland are prerequisites for the redistribution of REMS to short dark periods.
  In the golden hamster exposed to short light-dark cycles, REMS predominated during the light periods. Thus the enhancement of REMS by short periods of light or darkness extends to a further species other than the rat.

- The effect of serotonin agonists and antagonists on sleep and motor activity was studied. Serotonin, lysergic acid diethylamide (LSD), p-chlorophenylalanine (PCPA), tryptophan (TRP) and FU 29-245 (a serotonin antagonist) were applied either by the intraperitoneal, intravenous, or cerebroventricular route. Serotonin caused either arousal or had no effect on the vigilance states. FU 29-245 reduced REMS and enhanced the power of the EEG delta band. LSD, a mixed serotonin agonist and antagonist, had no effect on sleep. PCPA, which lowered serotonin concentrations in the brain, increased motor activity on the day after injection, and reduced sleep. The effect of PCPA on sleep could be reversed for a few hours by TRP which caused a temporary increase in the brain serotonin level.
- Similar experiments were carried out with drugs affecting the cholinergic system: physostigmine, atropine and carbachol. At high concentrations physostigmine had an arousing effect, while at lower concentrations it neither affected the amount of sleep states, nor REMS latency and REMS duration. Atropine pellets had no effect on total sleep time, although they slightly reduced REMS the day after the implantation. Rats infused with carbachol were either immediately aroused, or their vigilance state was not affected.
- The effect of Delta Sleep Inducing Peptide (DSIP) and arginine vasotocin (AVT) on motor activity and sleep was investigated in the rat. Motor activity in the first 4 h after systemic injection of DSIP was increased, and the power in the delta band after administration of DSIP and AVT into the third ventricle was reduced, indicating an unspecified biological action of these peptides. No evidence was found for a motor activity reducing effect or for a sleep inducing effect of either peptide. Thus neither DSIP nor AVT qualify as specific sleep-promoting substances.
- The effect of cerebrospinal fluid (depr.CSF) taken from sleep deprived donor rats and infused into the lateral ventricle of

sleeping recipient rats was studied. Total sleep time was slightly but significantly higher 2 and 3 hours after the infusion. Thus sleep deprivation may enhance the secretion of a hypnotoxin into the CSF in rats.

- On the basis of the present study it is concluded that the combination of physiological and pharmacological methods constitutes a reasonable and promising approach to the investigation of the mechanisms involved in sleep regulation.

VII REFERENCES

Aghajanian, G.K. and Haigler, H.J. (1975). Hallucinogenic indoleamines: preferential action upon presynaptic serotonin receptors. Psychopharmacol. Commun. 1, 619-629.

- and Weiss, T. (1968). LSD blocks the increase in rat brain serotonin turnover induced by an elevated ambient temperature. Nature 220, 795-796.

- Foote, W.E. and Sheard, M.H. (1968). Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe. Science 161, 706-708.

- Foote, W.E. and Sheard, M.H. (1970). Action of psychotogenic drugs on single midbrain raphe neurons. J. Pharmacol. exp. Ther. 171, 178-187.

- Graham, A.W. and Sheard, M.H. (1970). Serotonin-containing neurons in the brain: depression of firing by monoamine oxidase inhibitors. Science 169, 1100-1102.

- Bloom, F.E., Lovell, R.A., Sheard, M.H. and Freedman, D.X. (1966). The uptake of 5-hydroxytryptamine -3H from the cerebral ventricles: autoradiographic localization. Biochem. Pharmacol. 15, 401-408.

Amatruda, T.T., Black, D.A., McKenna, T.M., McCarley, R.W. and Hobson, J.A. (1975). Sleep cycle control and cholinergic mechanisms: differential effects of carbachol injections at pontine brain stem sites. Brain Res. 98, 501-515.

Aschoff, J., Figala, J. and Pöppel, E. (1973). Circadian rhythms of locomotor activity in the golden hamster (mesocricetus auratus) measured with two different techniques, J. Comp. Physiol. Psychol. 85, 20-28.

Axelrod, J. (1974). The pineal gland: a neurochemical transducer. Science 184, 1341-1348.

Bass, N.H. and Lundborg, P. (1973). Postnatal development of bulk flow in the cerebrospinal fluid system of the albino rat: clearance of carboxyl-(^{14}C) insulin after intrathecal infusion. Brain Res. 52, 323.

Baxter, B.L. (1969). Induction of both emotional behavior and a novel form of REM sleep by chemical stimulation applied to cat mesencephalon. Exp. Neurol. 23, 220-229.

Bennett, J.P. and Snyder, S.H. (1975). Stereospecific binding of D-lysergic acid diethylamide (LSD) to brain membranes: relationship to serotonin receptors. Brain Res. 94, 523-544.

Bentley, P.J. (1958). The effects of neurohypophysial extracts on water transfer across the wall of the isolated urinary bladder of the toad bufo marinus. J. Endocrin. 17, 201-209.

Bilkova, J., Radil-Weiss, T. and Bohdanecky, Z. (1971). The influence of low LSD dose administration during sleep in rats. Psychopharmacol. 20, 395-399.

Borbély, A.A. (1970). Telemetry of body temperature, motor activity, food and fluid intake in the rat. Proceedings of the Fourth International Congress on Pharmacology 5, 295-297.

- (1976). Sleep and motor activity of the rat during ultrashort light-dark cycles. Brain Res. 114, 305-317.

- (1978a). Effects of light on sleep and activity rhythms. Progress in Neurobiology 10, 1-31.

- (1978b). Active wakefulness and paradoxical sleep: common mechanisms? The Behavioral and Brain Sciences 305, 143-148.

- and Neuhaus, H.U. (1979). Sleep-deprivation: effects on sleep and EEG in the rat. J. Comp. Physiol. 133, 71-87.

- and Tobler, I. (1979). Cerebroventricular infusion in the rat: depression of motor activity and paradoxical sleep. Neurosci. Letts. 12, 75-80.

- Huston, J.P. and Waser, P.G. (1973). Physiological and behavioral effects of parachlorophenylalanine in the rat. Psychopharmacol. 31, 131-142.

- Huston, J.P. and Waser, P.G. (1975). Control of sleep states in the rat by short light-dark cycles. Brain Res. 95, 89-101.

- Neuhaus, H.U. and Tobler, I. (1980). Effect of p-chlorophenylalanine and tryptophan on sleep, EEG and motor activity in the rat. (in press)

Bourgoin, S., Artaud, F., Bockaert, J., Héry, F., Glowinski, J. and Hamon, M. (1978). Paradoxical decrease of brain 5-HT turnover by metergoline a central 5-HT receptor blocker. Arch. of Pharmacol. 302, 313-321.

Breisch, S.T., Zemlan, F.P. and Hoebel, B.G. (1976). Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. Science 192, 382-384.

Browman, L.G. (1953). The effect of controlled temperatures upon the spontaneous activity of the albino rat. J. exp. Zool. 94, 477-489.

Chamblin, M.H. and Drew, W.G. (1971). The effects of lights-off stimulation on the circadian distribution of REM sleep in the cat. Commun. Behav. Biol. 6, 111-114.

Chutkow, J.G. (1968). Magnesium and calcium in the cerebrospinal fluid of the rat. Proc. Soc. Exp. Biol. Med. 128, 555-558.

Cloetta, M. and Fischer, H. (1930). Ueber die Wirkung der Kationen Ca, Mg, Sr, Ba, K und Na bei intrazerebraler Injektion. Arch. Exper. Pathol. Pharmakol. 158, 254-281.

Coculescu, M., Serbanescu, A. and Temeli, E. (1979). Influence of arginine vasotocin administration on nocturnal sleep of human subjects. Waking and Sleeping 3, 1-5.

Costa, E. and Aprison, M.H. (1958). Distribution of intracarotidly injected serotonin in the brain. Amer. J. Physiol. 192, 95-100.

Cserr, H. (1965). Potassium exchange between cerebrospinal fluid, plasma and brain. Amer. J. Physiol. 209, 1219-1226.

Curzon, G. and Green, A.R. (1970). Rapid method for determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Br. J. Pharmacol. 39,653-655.

Delorme, F. (1966). Monoamines et sommeils; étude polygraphique neuropharmacologique et histoclinique des états de sommeil chez le chat. In: Thèse Université de Lyon, Imprimerie LMD, pp. 168.

- Froment, J.L. and Jouvet, M. (1966). Suppression du sommeil par la p.chlorométhamphetamine et la p.chlorophénylalanine. C.R. Soc. Biol. (Paris) 160, 2347-2351.

Demole, V. (1927). Pharmakologisch-anatomische Untersuchungen zum Problem des Schlafes. Arch. Exper. Pathol. Pharmakol. 120, 229.

Fencl, V., Koski, G. and Pappenheimer, J.R. (1971). Factors in cerebrospinal fluid from goats that affect sleep and activity in rats. J. Physiol. 216, 565-589.

Folk, G.E., jr. (1954). The effect of restricted feeding time on the diurnal rhythm of running activity in the white rat. Anat. Rec. 120, 786-787.

Fuxe, K. and Owran, C. (1965). Cellular localization of monoamines in the area postrema of certain mammals. J. Comp. Neurol. 125, 337-355.

- and Ungerstedt, U. (1968). Histochemical studies on the distribution of catecholamines and 5-hydroxytryptamine after intraventricular injections. Histochemie 13, 16-28.

Gal, E.M., Roggeveen, A.E. and Millard, S.A. (1970). Dl ($2^{14}C$) p-chlorophenylalanine as an inhibitor of tryptophan 5-hydroxylase. J. Neurochem. 17, 1221-1235.

Gisolfi, C.V., Wilson, N.C., Myers, R.D. and Phillips, M.I. (1976). Exercise thermoregulation: hypothalamic perfusion of excess calcium reduces elevated colonic temperature of rats. Brain Res. 101, 160-164.

Glowinski, J. and Iversen, L.L. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of (^{3}H) norepinephrine, (^{3}H) dopamine and (^{3}H) DOPA in various regions of the brain. J. Neurochem. 13, 655-659.

Goodrich, C.A., Greehey, B., Miller, T.B. and Pappenheimer, J.R. (1969). Cerebral ventricular infusions in unrestrained rats. J. appl. Physiol. 26, 137-140.

Green, R.A., Gillin, J.C. and Wyatt, R.J. (1976). The inhibitory effect of intraventricular administration of serotonin on spontaneous motor activity in rats. Psychopharmacol. 51, 81-84.

Green, W.J. (1969). LSD and the sleep-dream cycle. Exp. Med. Surg. 27, 138-144.

Haigler, H.J. and Aghajanian, G.K. (1974). Peripheral serotonin antagonists: failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. J. Neural. Transm. 35, 257-273.

Hamon, M., Bourgoin, S., Héry, F. and Simonnet, G. (1978). Activation of tryptophan hydroxylase by adenosine triphosphate, magnesium and calcium. Mol. Pharmacol. 14, 99-110.

Hanselmann G. and Borbély A.A. (1976). Response of rat brain indoles and motor activity to short light - dark cycles. J. of Neurochem. 26, 951-955.

Harrick, N.J. (1967). Internal reflection spectroscopy. Wiley Interscience, New York.

Hartmann, E. (1967). The effect of LSD and L-tryptophan on the sleep-dream cycle in the rat. Psychon. Sci. 8, 295-296.

- (1969). The biochemistry and pharmacology of the d-state (dreaming-sleep). Exp. Med. Surg. 27, 105-119.

Haupt, R. (1973). The sleep-wake behavior of rats. A comparison of cable and telemetric registration. In: Koella and Levin Sleep: Physiology, Biochemistry, Psychology, Pharmacology, Clinical Implications, pp. 285-289, Karger, Basel.

Hernández-Peón, R., O'Flaherty, J.J. and Mazzuchelli-O'Flaherty, A.L. (1967). Sleep and other behavioural effects induced by acetylcholinic stimulation of basal temporal cortex and striate structures. Brain Res. 4, 243-267.

Hobson, J.A. (1964). The effect of LSD on the sleep cycle of the cat. Electroenceph. clin. Neurophysiol. 17, 52-56.

- McCarley, R.W. and Wyzinski, P.W. (1975). Sleep oscillation reciprocal discharge by two brainstem neuronal groups. Science 189, 55-58.

Johnson, J.H. and Sawyer, C.H. (1971). Adrenal steroids and the maintenance of a circadian distribution of paradoxical sleep in rats. Endocrinology 89, 507-512.

- Adler, N.T. and Sawyer, C.H. (1970). Effects of various photoperiods on the temporal distribution of paradoxical sleep in rats. Exp. Neurol. 27, 162-171.

Jouvet, M. (1961). Telencephalic and rhombencephalic sleep in the cat. In: The nature of sleep. eds: Wolstenholme, G.E.W. and O'Conner, M., pp. 188-206, J. and A. Churchill, London.

- (1972). The role of monoamines and acetylcholine containing neurons in the regulation of the sleep-waking cycle. Ergebn. Physiol. 64, 166-307.

- (1977). Neuropharmacology of the sleep-waking cycle. Handbook of Psychopharmacol. 8, 233-293.

Kafi, S., Monnier, M. and Gaillard, J.-M. (1979). The delta-sleep inducing peptide (DSIP) increases duration of sleep in rats. Neurosci. Letts. 13, 169-172.

Kastin, A.J., Nissen, C., Schally, A.V. and Coy, D.H. (1978). Radioimmunoassay of DSIP-like material in rat brain. Brain Res. 3, 691-695.

Khazan, N. and Sawyer, C.H. (1964). Mechanisms of paradoxical sleep as revealed by neurophysiologic and pharmacologic approaches in the rabbit. Psychopharmacol. (Berlin) 5, 457-466.

Knott, P.J. and Curzon, G. (1974). Effect of increased rat brain tryptophan on 5-hydroxytryptamine and 5-hydroxyindolylacetic acid in the hypothalamus and other brain regions. J. Neurochem. 22, 1065-1091.

Koe, B.K. and Weissmann, A. (1966). P-chlorophenylalanine, a specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154, 499-516.

Koella, W.P. and Czicman, J.S. (1966). Mechanism of the EEG-synchronizing action of seretonin. Amer. J. Physiol. 211, 926-934.

- Feldstein, A. and Czicman, J.S. (1968). The effect of para-chlorophenylalanine on the sleep of cats. Electroenceph. clin. Neurophysiol. 25, 481-490.

Kolb, B. and Whishaw, I.Q. (1977). Effects of brain lesions and atropine on hippocampal and neurocortical electroencephalograms in the rat. Exp. Neurol. 56, 1-22.

König, J.F.R. and Klippel, R.A. (1963). The rat brain. Williams and Wilkins Company, Baltimore.

Kramis, R., Vanderwolf, C.H. and Bland, B.H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane and pentobarbital. Exp. Neurology 49, 58-85.

Kripke, D.F., Lavie, P., Parker, D., Huey, L. and Deftos, L.J. (1978). Plasma parathyroid hormone and calcium are related to sleep stage cycles. J. of Clin. Endocr. and Metabolism 47, 1021-1027.

Krueger, J.M., Pappenheimer, J.R. and Karnovsky, M.L. (1978). Sleep-promoting factor S: purification and properties. Proc. Natl. Acad. Sci. USA 75, 5235-5238.

Lisk, R.D. and Sawyer, C.H. (1966). Induction of paradoxical sleep by lights-off stimulation. Proc. Soc. exp. Biol. (N.Y.) 123, 664-667.

Lucas, E.A. (1979). Effects of a Short Light-Dark Cycle on the Sleep-Wake Patterns of the Cat. Sleep 1, 299-317.

Mendelson, W.B., Gillin, J.C., Pisner, G. and Wyatt, R.J. (1980). Arginine vasotocin and sleep in the rat. Brain Res. (in press)

Monnier, M. and Hösli, L. (1964). Dialysis of sleep and waking factors in blood of the rabbit. Science 146, 796-798.

- and Tissot, R. (1958). Action de la réserpine et de ses médiateurs (5-HTP-serotonine et DOPA-noradrenaline) sur le comportement et le cerveau du lapin. Helv. Physiol. Acta 16, 255-267.

- Dudler, L., Gächter, R. and Schoenenberger, G.A. (1977). Delta sleep-inducing peptide (DSIP): EEG and motor activity in rabbits following intravenous administration. Neurosci. Letts. 6, 9-13.

Mouret, J., Bobillier, P. and Jouvet, M. (1968). Insomnia following p-chlorophenylalanine in the rat. European J. Pharmacol. 5, 17-22.

- Froment, J.L., Bobillier, P. and Jouvet, M. (1967). Etude neuropharmacologique et biochimique des insomnies provoquées par la p-chlorophenylalanine. J. Physiol. (Paris) 59, 463-464.

Muzio, J.N., Howard, M., Roffwarg, H.P. and Kaufman, E. (1966). Alterations in the nocturnal sleep cycle resulting from LSD. Electroenceph. clin. Neurophysiol. 21, 313-323.

Myers, R.D. (1972). Methods for perfusing different structures of the brain. In: Methods in Psychobiology, Vol. 2. ed: Myers, R.D., p. 169, Academic Press, London.

- and Brophy, P.D. (1972). Temperature changes in the rat produced by altering the sodium-calcium ratio in the cerebral ventricles. Neuropharmacol. 11, 351-361.
- and Melchior, C.L. (1976). Feeding and body temperature in the rat: diencephalic localization of changes produced by excess calcium ions. Brain Res. Bull. 1, 33.
- Evans, J.E. and Yaksh, T.L. (1972). Ethanol preference in the rat: interactions between brain serotonin and ethanol, acetaldehyde, paraldehyde, 5-HTP and 5-HTOL. Neuropharmacol. 11, 539.
- Veale, W.L. and Beleslin, D.B. (1970). Calcium evoked release of 5-hydroxytryptamine from the brain of the unanaesthetized cat. Experimentia 26, 1324.
- Bender, S.A., Krstić, M.K. and Brophy, P.D. (1972). Feeding produced in the satiated rat by elevating the concentration of calcium in the brain. Science 176, 1124.

Nagasaki, H., Iriki, M., Inoué, S. and Uchizono, K. (1974). The presence of a sleep-promoting material in the brain of sleep-deprived rats. Proc. Japan Acad. 50, 241-247.

Neuhaus, H.U. and Borbély, A.A. (1978). Sleep telemetry in the rat. II. Automatic identification and recording of vigilance states. Electroenceph. clin. Neurophysiol. 44, 115-119.

O'Hare, M.J. and Nice, E.C. (1979). Hydrophobic high-performance liquid chromatography of hormonal polypeptides and proteins on alkylsilane-bonded silica. J. of Chromatography 171, 209-226.

Oliverio, A. and Malorni, W. (1979). Wheel running and sleep in two strains of mice: plasticity and rigidity in the expression of circadian rhythmicity. Brain Res. 163, 121-133.

Palaič, D., Page, I.H. and Khairallah, P.A. (1967). Uptake and metabolism of (^{14}C) serotonin in rat brain. J. of Neurochem. 14, 63-69.

Pappenheimer, J.R., Koski, G., Fencl, V., Karnovsky, M.L. and Krueger, J. (1975). Extraction of sleep-promoting factor S from cerebrospinal fluid and from brains of sleep-deprived animals. J. Neurophysiol. 38, 1299-1311.

Pavel, S. (1978). Arginine vasotocin as a pineal hormone. J. of Neural Transmission 13, 135-155.

- Psatta, D. and Goldstein, R. (1977a). Slow-wave sleep induced in cats by extremely small amounts of synthetic and pineal vasotocin injected into the third ventricle of the brain. Brain Res. Bull. 2, 251-254.

- Cristoveanu, A., Goldstein, R. and Calb, M. (1977b). Inhibition of release of corticotropin releasing hormone in cats by extremely small amounts of vasotocin injected into the third ventricle of the brain. Evidence for the involvement of 5-hydroxytryptamine-containing neurons. Endocrinology 101, 672-678.

- Ghinea, E., Goldstein, R. and Matulevičius, V. (1978). Vasotocin biosynthesis by cultured pineal glands from adult male rats. J. Endocr. 77, 147-148.

- Luca, N., Calb, M. and Goldstein, R. (1979). Inhibition of release of luteinizing hormone in the male rat by extremely small amounts of arginine vasotocin: further evidence for the involvement of 5-hydroxytryptamine-containing neurons in the mechanism of arginine vasotocin. Endocrinology 104, 517-523.

- Goldstein, R., Popoviciu, L., Corfariu, O., Földes, A. and Farkas, E. (1980). Pineal vasotocin: REM sleep dependent release into cerebrospinal fluid of man. Waking and Sleeping (in press).

Pellegrino, L.J. and Cushman, A.J. (1967). A stereotaxic atlas of the rat brain. Meredith Publishing Company, New York.

Piéron, H. (1913). Le problème physiologique du sommeil. Paris: Masson, pp. 520.

Polc, P., Schneeberger, J. and Haefely, W. (1978). Effect of the delta sleep-inducing peptide (DSIP) on the sleep-wakefulness cycle of cats. Neurosci. Letts. 9, 33-36.

Rechtschaffen, A., Dates, R., Tobias, M. and Whitehead, W.E. (1969). The effects of lights-off stimulation on the distribution of paradoxical sleep in the rat. Commun. Behav. Biol., Part A 3, 93-99.

- Lovell, R.A., Freedman, D.X., Whitehead, W.E. and Aldrich, M. (1973). The effect of parachlorophenylalanine on sleep in the rat: some implications for the serotonin-sleep hypothesis. In: Serotonin and Behavior. eds: Barchas, J. and Usdin, E., pp. 401-424, Academic Press, New York.

Reed, D.J., Withrow, C.D. and Woodbury, D.M. (1967). Electrolyte and acid-base parameters of rat cerebrospinal fluid. Exp. Brain Res. 3, 212-219.

Ruedin, P., Bisang, J., Waser, P.G. and Borbély, A.A. (1978). Sleep telemetry in the rat. I. A miniaturized FM-AM transmitter for EEG and EMG. Electroenceph. clin. Neurophysiol. 44, 112-114.

Sachs, J., Ungar, J., Waser, P.G. and Borbély, A.A. (1976). Factors in cerebrospinal fluid affecting motor activity in the rat. Neurosci. Letts. 2, 83-86.

Sadtlers Standard Spectra (1970). Heyden, London.

Sandoz Ltd. (1977). Pharmacological exposé.

Satinoff, E., Adler, N., Kraus, H. and Flammino, F. (1975). Paradoxical sleep duration during lights-off and lights-on in ground squirrels. Physiol. Behav. 15, 631-632.

Scherschlicht, R., Schneeberger, J., Steiner, M. and Haefely, W. (1979). Delta sleep inducing peptide (DSIP) antagonizes morphine insomnia in cats. Sleep Res. 8,

Schoenenberger, G.A., Maier, P.F., Tobler, H.J. and Monnier, M. (1977). A naturally occurring delta-EEG enhancing nonapeptide in rabbits. Pflügers Arch. 369, 99-109.

- Maier, P.F., Tobler, H.J., Wilson, K. and Monnier, M.(1978). The delta EEG (sleep)-inducing peptide (DSIP). Pflügers Arch. 376, 119-129.

Seoane, J.R. and Baile, C.A. (1973). Ionic changes in cerebrospinal fluid and feeding, drinking and temperature of sheep. Physiol. Behav. 10, 915-923.

Sitaram, N., Mendelson, W.B., Wyatt, R.J. and Gillin, J.C. (1977). The time-dependent induction of REM sleep and arousal by physostigmine infusion during normal human sleep. Brain Res. 122, 562-567.

- Wyatt, R.J., Dawson, S. and Gillin, J-C. (1976). REM sleep induction by physostigmine infusion during sleep. Science 191, 1281-1282.

Spiegel, R. (1979). Manuscript on effect of FU 29-245 on sleep of healthy subjects.

Steigrad, P., Tobler, I., Waser, P.G. and Borbély, A.A. (1978). Effect of p-chlorophenylalanine on cerebral serotonin binding, serotonin concentration and motor activity in the rat. Arch. of Pharmacol. 305, 143-148.

Stern, W., Geiger, E. and Jacobs, B. (1976). Impaired regulation of REM sleep by light: dark cycles in hypophysectomized rats. Sleep Res. 5, 244.

Toszeghi, P., Tobler, I. and Borbély, A.A. (1978). Cerebral ventricular infusion of excess calcium in the rat: effects on sleep states, behavior and cortical EEG. European J. Pharmacol. 51, 407-416.

Toyoda, J., Sasaki, K. and Kurihara, M. (1967). A polygraphic study on the effect of atropine on human nocturnal sleep. Folia Psychiatr. Neurol. Jap. 20, 275-289.

Trulson, M.E., Ross, C.A. and Jacobs, B.L. (1976). Behavioral evidence for the stimulation of CNS serotonin receptors by high doses of LSD. Psychopharmacol. Commun. 2, 149-164.

Valatx, J.L., Chouvet, G. and Jouvet, M. (1975). Sleep-waking cycle of the hypophysectomized rat. Prog. Brain Res. 42, 115-120.

- Roussel, B. and Curé, M. (1973). Sommeil et température cérébrale du rat au cours de l'exposition chronique en ambiance chaude. Brain Res. 55, 107-122.

Van Twyver, H. (1969). Sleep patterns of 5 rodent species. Physiol. Behav. 4, 901-905.

- Webb, W., Dube, M. and Zackheim, M. (1973). Effects of environmental and strain differences on EEG and behavioral measurement of sleep. Behav. Biol. 9, 105-110.

Veale, W.L. and Myers, R.D. (1971). Emotional behavior, arousal and sleep produced by sodium and calcium ions perfused within the hypothalamus of the cat. Physiol. Behav. 7, 601.

Webb, W. and Friedman, J. (1971). Attempts to modify the sleep patterns of the rat. Physiol. Behav. 6, 459-460.

Results which have been previously published:

Borbély, A.A. and Tobler, I. (1979). Cerebroventricular infusion in the rat: depression of motor activity and paradoxical sleep. Neurosci. Letts. 12, 75-80.

- Neuhaus, H.U. and Tobler, I. (1980). Effect of p-chlorophenylalanine and tryptophan on sleep, EEG and motor activity in the rat. Behav. Brain Res. In press.

- Steigrad, P. and Tobler, I. (1980). Effect of sleep deprivation on brain serotonin in the rat. Behav. Brain Res. 1, 205-210.

- Tobler, I. and Steigrad, P. (1979). Chronic drug administration in behavioral studies by subcutaneous pellets. Pharmac. Ther. 5, 451-453.

Steigrad, P., Tobler, I., Waser, P.G. and Borbély, A.A. (1978). Effect of p-chlorophenylalanine on cerebral serotonin binding, serotonin concentration and motor activity in the rat. Arch. of Pharmacol. 305, 143-148.

Tobler, I. and Borbély, A.A. (1977). Enhancement of paradoxical sleep by short light periods in the golden hamster. Neurosci. Letts. 6, 275-277.

- and Borbély, A.A. (1978). Short light-dark cycles and paradoxical sleep in the rat: effect of strain difference and hypophysectomy. Behav. Biol. 23, 395-398.

- and Borbély, A.A. (1980). Effect of Delta Sleep Inducing Peptide (DSIP) and Arginine Vasotocin (AVT) on Sleep and Motor Activity in the Rat. Waking and Sleeping 4, 139-153.

Toszeghi, P., Tobler, I. and Borbély, A.A. (1978). Cerebral ventricular infusion of excess calcium in the rat: effects on sleep states, behavior and cortical EEG. Europ. J. of Pharmacol. 51, 407-416.

CURRICULUM VITAE

Irene Maria Tobler-Kost

Born: October 17, 1946

Place of birth: Zurich, Switzerland

Place of origin: Teufen, Appenzell, Switzerland

Education

1952 - 1958	Lowerschool Pestalozzi, Lima, Peru
1958 - 1959	Middleschool Calvert course, Maria Elena, Chile
1959 - 1960	Middleschool Sta. Maria, Antofagasta, Chile
1960 - 1964	Middleschool and Highschool, Santiago College, Santiago, Chile
1964 - 1966	Cantonal Highschool, Zurich, Switzerland
1966 - 1971	University of Zurich, Biology (Major in Zoology)

Degrees

1966	Baccalaureate (Mat. B)
1971	University degree in natural sciences
1974	Highschool teaching diploma for biology and chemistry

Positions

1971 - 1978	Teaching in biology and chemistry at the Kantonsschule Riesbach, Zurich
1976 - 1980	Research assistant under Prof. Dr. A.A. Borbély at the Institute of Pharmacology, University of Zurich

Diese Dissertation entstand unter der Leitung von Herrn Professor Dr. A.A.Borbély am Pharmakologischen Institut der Universität Zürich, wo ich seit 1976 als Assistentin tätig bin.

Während meines Studiums besuchte ich Vorlesungen, Uebungen und Praktika bei den folgenden Dozenten:

K.Akert, J.Biegert, A.Borbély, H.Burla, P.S.Chen, C.Cook, C.Eugster, E.Hadorn, H.Hediger, R.Hunsperger, H.Jungen, S.Kubik, H.Kummer, W.Lichtensteiger, R.Nöthiger, H.Oswald, D.Rast, O.Rohweder, H.Rüst, A.Rutishauser, J.Schlittler, B.Stüssi, P.Tardent, E.Thomas, G.Tönduri, G.Wagner, B.van der Waerden, H.Wanner, R.Wehner, Ch.Weissmann und V. Ziswiler.